



This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

- + *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.
- + *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.
- + *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.
- + *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at <http://books.google.com/>



Chem 499.06

Harvard College Library



FROM THE BEQUEST OF

DANIEL TREADWELL

Rumford Professor and Lecturer on the Application
of Science to the Useful Arts
1834-1845

SCIENCE CENTER LIBRARY

CHEMISTRY OF THE ALBUMENS

CHEMISTRY OF THE ALBUMENS

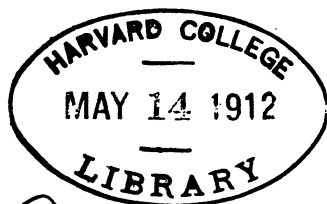
TEN LECTURES DELIVERED IN THE MICHAELMAS
TERM, 1904, IN THE PHYSIOLOGICAL DEPARTMENT
OF UNIVERSITY COLLEGE, LONDON

BY S. B. SCHRYVER, D.Sc. (LOND.), Ph.D. (LEIPZIG)
LECTURER IN PHYSIOLOGICAL CHEMISTRY TO UNIVERSITY COLLEGE, LONDON

PUBLISHED UNDER THE AUSPICES OF THE UNIVERSITY
OF LONDON

LONDON
JOHN MURRAY, ALBEMARLE STREET, W.
1906

Chem 499.06



Treadwell fund

P R E F A C E

THE following lectures were given with the object of summarising the methods that have been employed in the investigations of the chemical structure of the albumens, and the results obtained. The structure of the albumens is only one of the problems presented to the biological chemist. In this branch of study, considerable progress has been made during the last few years.

An equally important study, however, which is still in its infancy, relates to the chemical dynamics of the albumen molecules when carrying out their normal functions in the living tissue. Attention is called to this branch of biological chemistry in the very incomplete review of the theories of biochemical action given in Lectures IX. and X. When few facts are known, we are obliged to content ourselves with theories; if the latter, however, stimulate subsequent research, they will justify their existence. For this reason I have not hesitated to include Lectures IX. and X. in this publication, imperfect as I feel they are.

I am indebted to Dr R. H. Aders Plimmer for his kindness in revising the proofs.

S. B. S.

November 1905.

CONTENTS

LECTURE	PAGE
I. THE GENERAL PROPERTIES AND REACTIONS OF THE ALBUMENS	I
II. THE DEGRADATION PRODUCTS OF THE ALBUMENS .	18
III. THE DEGRADATION PRODUCTS OF THE ALBUMENS— <i>continued</i>	42
IV. THE PROTAMINES AND HISTONES	65
V. THE DEGRADATION PRODUCTS OF NUCLEIC ACID .	79
VI. THE CONSTITUTION OF THE NUCLEIC ACIDS. THE GLYCO-PROTEIDS	103
VII. THE CHEMISTRY OF HÆMOGLOBIN AND ITS CHROMATOGENIC GROUP	121
VIII. THE INTERMEDIARY DEGRADATION PRODUCTS OF THE ALBUMENS; THE SYNTHESIS OF THE POLYPEPTIDES	139
IX.-X. GENERAL THEORIES OF BIOCHEMICAL ACTION . .	167
APPENDIX	187
INDEX	189

ABBREVIATIONS IN THE LITERATURE REFERENCES

A.P.P. Archiv für Experimentelle Pathologie und Pharmacologie
(Schmiedeberg).

Ber. Berichte der deutschen chemischen Gessellschaft.

H.Z. Zeitschrift für physiologische Chemie (Hoppe-Seyler).

CHEMISTRY OF THE ALBUMENS

LECTURE I

THE GENERAL PROPERTIES AND REACTIONS OF THE ALBUMENS

THE investigation of the chemical mechanism of a living body is analogous to the investigation of a complex machine in working order performing certain definite functions. Our researches on such a mechanism can proceed in two directions; either, we can investigate the construction of the machine, or we can determine its functions and its needs, the fuel it requires, its waste products, etc. Our researches on the albumen in the living organism can likewise branch out into two different directions. We can investigate the construction of the albumen molecule, which is known to perform certain vital functions in the living body, or we can proceed to determine these functions. It is to the consideration of the first of these problems that the following lectures will be devoted. The determination of the construction of the albumen molecule is essentially a task for the chemist, and it will be my endeavour in the course of the following lectures to demonstrate the methods that have been employed in the investigations on the constitution of the albumens, and to give a short account of the principal results that have been obtained up to the present time.

At the outset, however, we encounter certain difficulties. Let us refer again to our analogy of the working machine. It is conceivable that we are so ignorant of mechanics as to be unable to remove our machine from its working position *in*

A

situ, without considerable damage to its more delicate parts; it is impossible to remove an albumen from its position in the living body without subjecting it to a certain amount of damage. Still, in spite of this, we can, by our somewhat crude laboratory methods, gain a certain amount of insight into its general construction, and ascertain to a very great extent the parts of which it is composed, although we are at the present day still far from being able to reconstruct the machine from those parts, and so synthesise an albumen.

We will now proceed to investigate the general properties of an albumen.

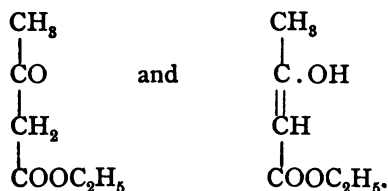
As an example, we might take the white of egg as a product which is an easily accessible type of an albumen; this substance, "Eiweiss," is, in fact, the generic name in German for the whole class of albumens.

A filtered solution of egg albumen in water has certain properties which can be readily demonstrated. On heating, the albumen is rendered insoluble—coagulation takes place. This illustrates the readiness with which a change takes place in a "natural albumen," as we shall term the body existing in a normal functioning state in a living organism. Furthermore, the organic matter in the solution is incapable of passing through vegetable parchment. We are dealing therefore with a colloidal solution, and therefore with a solution of a body of very high molecular weight. We shall at the present moment, however, neglect other physical properties, for reasons which will be evident when we come to consider one of the first chemical properties to which a chemist generally turns his attention, viz., the property of functioning as an acid, base, or neutral body.

We shall find, then, that it is possible to add minute quantities of alkali, or of acid, without destroying the original "amphoteric" character of the solution when tested with litmus paper. This property of functioning as a weak acid, or a weak base, is characteristic, as Cohnheim has pointed out in the case of the albumens, of those classes of bodies known as pseudo-acids and pseudo-bases. Our knowledge of these classes of bodies we

owe principally to the researches of Hantzsch, whose work it will be necessary to consider in this place, on account of its general bearings on the chemistry of the albumens.

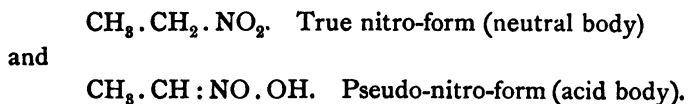
It has for long been known that certain bodies containing the group $\text{CH}_2\text{.CO}$ can act as if the molecule were capable of transformation under different circumstances, and could exist in two forms, owing to the presence of a labile hydrogen atom. Of this class of bodies, the best-known example is perhaps ethyl acetoacetate, which can exist in the two forms.



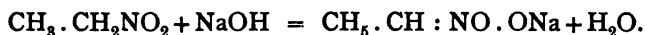
The former is called the ketonic, and the latter the enolic form. Such bodies are now generally known as "dynamic isomers."

A special case of such isomerism exists when one of the isomers is of acidic character and the other neutral, or when one isomer is of basic character and the other neutral. These cases have formed the special subject of Hantzsch's researches.

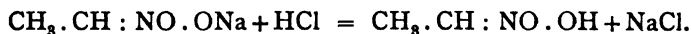
It has been found by Hantzsch(3) that the nitro-paraffins which are neutral, non-conducting bodies in their original state, are capable of *slowly* neutralising caustic soda. If, now, an equivalent quantity of acid be added to such a neutralised solution, and the electric conductivity be measured, it will be found at first to be greater than that of sodium chloride solution alone. On standing, however, the conductivity of such a solution diminishes, until finally it sinks to that of sodium chloride. Hantzsch explains this result by assuming that the nitro-paraffin is capable of existing in two forms, viz. (in case of nitroethane),



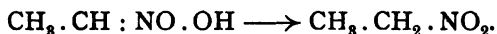
In the presence of alkali, the true nitro-form is slowly converted into the pseudo-form—



When, however, the sodium salt of the pseudo-nitroethane is treated with hydrochloric acid, the following reaction takes place—



This solution has at first the electric conductivity of the pseudo-acid and sodium chloride. On standing, however, the conductivity diminishes, until finally it sinks to that of sodium chloride alone. The pseudo-nitro-form has been slowly changing into the non-conducting (non-ionised) neutral nitro-body,



The higher the temperature, the more rapidly such a change takes place.

A body like nitroethane, which can be converted under certain conditions into an acid form, is termed by Hantzsch a *pseudo-acid*.

The following are a few of the principal characteristics of a pseudo-acid :—

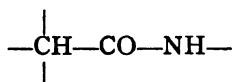
1. When an equimolecular quantity of an alkali is added to a pseudo-acid, neutralisation does not follow immediately, as would happen when an alkali is added to a true acid, but requires a certain amount of time.
2. Certain abnormalities in the electrolytic conductivity can be observed, such as those described above in connection with nitroethane.
3. Sometimes colour changes can be observed. Nitro-form, $\text{HC}(\text{NO}_2)_3$ for example, is a colourless body. But on treatment with alkalis it yields coloured salts. The ions of the pseudo-form are coloured.
4. Sometimes the two forms give different chemical reactions, *e.g.*, in some cases phosphorus pentachloride (PCl_5) will act with the pseudo-acid forming the acidyl chloride, but not with the isomer. Ammonia will form an addition product

directly with acids, either in a dry state or in a non-ionising medium. Ammonia will only form an addition product with a pseudo-acid in the presence of water.

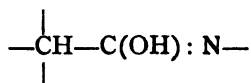
In some cases both the pseudo-form and its isomer can be actually isolated. In other cases the pseudo-form cannot be isolated in a free state.

Just as pseudo-acids have been shown to exist, so Hantzsch has also demonstrated the existence of analogous bodies of pseudo-basic character. Just as nitroethane in presence of *alkalis* tends to undergo an isomeric change with the formation of salts, so certain bodies in presence of *acids* tend to form salts under analogous conditions. Such bodies also exhibit abnormal neutralisation phenomena. It is not necessary to consider in greater detail their properties now.

It is probable that some albumens also have this pseudo-acid or pseudo-basic character, from the fact that weak acids or weak alkalis can be added to their solutions without producing a distinct acid or basic reaction. Further, as we shall see later on, albumens most probably contain the group



which is also capable of existing in the form



(the lactam and lactim forms), of which the former is of basic and the latter of acidic character. Hence it is possible that isomeric changes can take place in the albumen molecule into one or other of these forms, according to the character of the other bodies in solution.

Now, when we consider the fact that the albumen molecule is of enormous size, a relatively very small amount of an acid or of a base can act on a large amount of an albumen. When, furthermore, very small amounts of such bodies can cause

changes in the albumen molecule, which have a large influence on the physical properties, such as conductivity, etc., it is evident that the determination of a reliable physical constant in the case of the albumens is a matter attended with considerable difficulty—especially when we consider the additional difficulty of eliminating small traces of electrolytes. For this reason the older data on the physical properties of the albumens require in most cases revision, and will not be discussed in detail in this place. The possibility of the existence of the albumen molecule in both acid and basic form must also be taken into account in histological work, and for this reason great precautions must be exercised in drawing conclusions as to the chemical nature of structures from the capacity for combining with acidic or basic staining reagents.

It must be remembered, however, that all albumens do not possess the amphoteric character described above for egg albumen. Some, as we shall see later on, owing to the preponderance of true acidic groups, such as the nucleo-proteids, have a distinctly acid character; others, owing to preponderance of basic groups, such as the protamines, have a distinctly alkaline character.

We will now proceed to discuss the principal chemical reactions of the albumens.

As in certain conditions they may react as acids, we shall expect them to form salts. Hence the first class of reactions, viz,—

I.—THE REACTIONS OF THE METALLIC SALTS

The principal saline precipitants are the following:—

Ferric chloride.	Lead acetate.
Copper sulphate.	Zinc acetate
Mercuric chloride.	

These bodies give true metallic precipitates (*i.e.*, precipitates containing the metals), and are to be distinguished from the saline precipitants to be described below.

II.—ALKALOIDAL PRECIPITANTS

In view of the fact that the albumens are pseudo-basic, a mineral acid should be present, in order that the albumen should exist in the basic form. Hence the alkaloidal precipitant is employed in the presence of hydrochloric acid. This statement does not apply, however, to all albumens, and some, such as the protamines, are, owing to preponderance of basic groups, already so alkaline that the addition of free mineral acids is unnecessary.

The following are the principal alkaloidal precipitants employed :—

- (a) Phosphotungstic acid.
- (b) Phosphomolybdic acid.
- (c) Tannic acid.
- (d) Potassium iodide.
- (e) Potassio-mercuric iodide.
- (f) Potassium ferrocyanide and acetic acid. (Hydroferrocyanic acid.)
- (g) Trichloroacetic acid.
- (h) Picric acid. (Generally a mixture of picric and citric acids, or Esbach's reagent.)

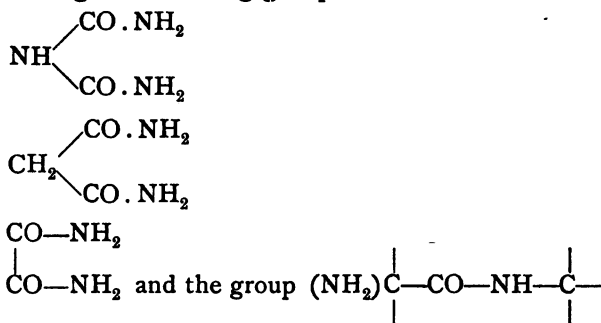
In addition to the above reagents, nitric acid and alcohol are sometimes used as precipitants. Alcohol is apt, however, to cause coagulation of the albumen, and nitric acid is only used in a limited number of cases. None of the above reagents can be used, however, if it is required to precipitate the albumen free from mixtures of foreign substances. In these cases the saline precipitants to be described in detail below are employed.

III.—COLOUR REACTIONS OF THE ALBUMENS

Various colour reactions have been described from time to time, and they are of importance in so far that we can in most cases draw some conclusion as to the group present in the molecule giving rise to the reaction. The significance of each reaction is in each case mentioned.

(a) The Biuret Reaction.

When, to a solution of an albumen, made strongly alkaline with caustic soda or potash, copper sulphate is added, drop by drop, a colour (pink to violet) is produced. This reaction has been investigated by Schiff, who finds that it is characteristic of bodies containing the following groups.



Krukenberg thinks that the pure albumens do not yield this reaction.

(b) The Xantho-Proteic Reaction.

Albumens give with nitric acid on boiling, either a yellow flocculent precipitate, or a yellow solution. On addition of excess of caustic alkali, or ammonia, the colour becomes orange-yellow.

This reaction has been investigated by Salkowski, who ascribes it to the presence of phenyl groups.

(c) Millon's Reagent.

This reagent is a solution of mercuric nitrate in water, containing free nitrous acid. On addition of an albumen, either in solution or in suspension, to Millon's reagent, and warming, a colour varying from pink to dark red is produced, both in the precipitate and in the supernatant liquid.

This reaction is characteristic, according to Salkowski, of bodies containing the oxyphenyl group, such as tyrosine.

(d) The Lead Sulphide Reaction.

On warming an albumen with caustic soda solution in the presence of a lead salt, such as lead acetate, a black colouration

is produced, indicating the presence of sulphur in the molecule. This reaction is produced, even by minute quantities of sulphur, and the depth of colouration produced gives some rough indication of the amount of sulphur in the body under investigation.

(e) *The various Reactions indicating the presence of Carbohydrate Groups in the Albumen Molecule.*

(i) *The Reaction of Molisch-Udransky.*—On the addition of concentrated sulphuric acid to an albumen solution to which a few drops of an alcoholic solution of α -naphthol have been added, a violet colour is produced, which turns yellow on addition of alcohol, ether, or caustic soda. If thymol be used instead of α -naphthol, a carmine red colour is produced. This reaction is due to the production of furfural by the action of the concentrated acid on the carbohydrate, and was originally discovered by Molisch. It was first applied to the carbohydrates by Seegen, and has been the subject of an exhaustive study by v. Udransky. The reaction must be employed with care, and conclusions can be drawn as to the relative amount of carbohydrate present in the molecule by the depth of colour produced.

(ii) *Bial's (1) Modification of the Orcine Reaction.*—This reaction is carried out in the following way: A small quantity of the dried albumen (enough to cover the point of a knife) is added to about 5 c.c. fuming hydrochloric acid, and the mixture is then warmed. The albumen is thereby nearly all dissolved. A little solid orcin is then added on the point of a knife, and then a drop of ferric chloride solution. After warming this mixture for some minutes a green colouration is produced, which is soluble in amyl alcohol. A conception as to the amount of carbohydrate groups present can be formed by the spectroscopic measurement of the amyl alcohol solution.

The reaction is also applicable to glucosamine, if the solution of this body be previously treated with nitrous acid. This reaction is important, as we shall see later on that it is in the form of glucosamine that the scission of the carbohydrate group takes place in the case of many of the albumens.

(iii) *Ehrlich's Dimethylaminobenzaldehyde Reaction*.—This reaction has been applied to the detection of carbohydrate groups in albumens. It is of minor importance, however, as colour reactions are given by other groups with this reagent.

(f) *The Reaction of Adamkiewicz as modified by Hopkins and Cole* (5).

The original reaction of Adamkiewicz was carried out in the following manner. Acetic acid was added to the albumen, and then concentrated sulphuric acid. At the junction of the liquids a violet colour was produced. It was found by Hopkins and Cole, however, that this colour reaction was produced only by certain samples of acetic acid. On investigating the causes of discrepancy in the results, they found that only those samples of glacial acetic acid which had remained standing for some time in the sunlight were capable of producing the colouration. In these cases the glacial acetic acid was found to have become oxidised, and contained small quantities of glyoxylic acid $\text{CHO} \cdot \text{COOH}$, and it was to this latter body and not to the acetic acid itself that the Adamkiewicz reaction was due. They therefore substituted for acetic acid a solution of glyoxylic acid, which is prepared by reducing a concentrated solution of oxalic acid with sodium amalgam.

This reaction is due to the presence in the albumen molecule of a body called tryptophane, the constitution of which will be discussed in the next lecture.

(g) *Liebermann's Reaction*.

Dry albumen which has been carefully freed from fat, gives on heating with fuming hydrochloric acid a deep blue to blue violet reaction. This colouration is also probably due to tryptophane. It is, however, of minor importance.

The Separation of Mixtures of Albumens.

It has been already stated that a solution of an albumen alters on heating. The ordinary methods of separating mixtures of organic compounds is not, therefore, applicable to the

albumens. We cannot, for example, separate entities from a mixture by fractional crystallisation or distillation. As colloidal bodies, however, they can be precipitated from solution by the addition of crystalloids. The ease with which an albumen can be "salted out" from solution by the addition of an inorganic salt varies very considerably with different products. Some classes of albumens can be "salted out" very readily, when the solution is only very partially saturated with a salt. Others require a much higher concentration of crystalloid before they separate from solution.

On the other hand, the power of precipitating an albumen from a solution varies very greatly with the different salts. Some salts precipitate at a much lower degree of concentration than others. The salts which are generally employed for salting out albumens may be divided roughly into three classes.

CLASS I. Sodium chloride.
Sodium sulphate.
Sodium acetate.
Sodium nitrate.
Magnesium sulphate.

CLASS II. Potassium acetate.
Calcium chloride. { These two salts are seldom used,
Calcium nitrate. { as they tend to render the pre-
cipitated albumen insoluble.

CLASS III. Ammonium sulphate.
Zinc sulphate.

The salts of the first class require a much higher concentration for the precipitation of albumens than those of the second. Those of the second, on the other hand, require a higher concentration than those of the third. The technique of the use of the various reagents was studied originally by Kühne and his pupils. In late years the systematic separation of albumens by the method of "salting out" has been studied very exhaustively by Hofmeister and his pupils, especially

Pick (8) and Zunz (9). The degree of concentration for any salt necessary for the precipitation of any particular albumen is characteristic for that body. Furthermore, in comparing two albumens with one another it will be found that if one is more readily precipitated than the other by one class of salts, it will also be more readily precipitated by the other classes. If, for example, one albumen is precipitated by a lower degree of concentration of sodium chloride than another albumen, it will also require a lower degree of concentration of zinc or ammonium sulphate. It is also possible, when comparing two albumens, to find that one is not precipitated at all by the salts in Class I., whereas the other is. It will follow that the former will require a higher degree of concentration of zinc or ammonium sulphate for precipitation than the latter. The limits between which precipitation commences and finishes on addition to an albumen of a salt, expressed as percentages of complete saturation, are numbers characteristic for each albumen.

An interesting example of the application of this method is given in Table I. Witte's peptone is a product obtained from fibrin by digestion with pepsine. By this means the original albumen is degraded by hydrolysis into simpler products, which are still of albuminous nature. It will be seen on referring to the table, that a partial separation at any rate has taken place, and that the different fractions obtained show differences when tested with the ordinary albumen reagents. One fraction, for example, the so-called glykalbumose fraction, shows very markedly the Molisch-Udransky reaction, which indicates that the greater part of the carbohydrate group is bound up with this fraction. Other fractions show the lead sulphide reaction very distinctly, a fact which indicates that in this fraction a large part of the sulphur bodies are bound up.

The importance of a separation of this description will be evident, when we come to consider in greater detail the constitution of an albumen molecule, for it should lead to some knowledge as to the method by which the different groups, of

TABLE I.—*Pick's Fractional Precipitation of Witte's Peptone.*

Fraction.	Precipitation Limits for Ammonium Sulphate in per- centages of full saturation.	Albumose.	Solubility in Alcohol.	Composition.					Biuret reaction.	Millon's reaction.	Xantho-proteic reaction.	Skatol reaction. (Potash melt.)	Mollisch reaction.	Lead Sulphide reaction.
				C.	H.	N.	S.	O.						
Hetero, Prot. albumose Fraction	24-42	Hetero albumose Prot. albumose	insol. in 32%	55.12	6.61	17.98	1.22	19.07	+	very weak	+	very weak	O	+
			sol. in 80%	55.64	6.80	17.66	1.21	18.69	+	strong	+	++	O	+
A. Fraction	54-62	Thio- albumose Small S. free fraction	insol. in 60-70%	48.96	6.90	16.02	2.97	25.15	+	+	+	+	O	+
			sol. in 70%	...	...	...	...	...	...	...	...	...	...	...
B. Fraction	70-95	B. I. fraction Glyk- albumose Other small fractions insoluble in alcohol	insol. in 35% (small fraction)	...	...	...	...	...	...	...	...	...	...	...
			insol. in 60-70%	48.72	7.03	13.76	30.49	...	+	+	+	weak	+	+
C. Fraction	100 % + acid	C. albumose	sol. in 67-80%	...	...	...	...	...	...	...	...	...	...	...
				34.52	5.85	17.24	42.89	...	+	O or very weak	++	O or very weak	O	O

Abbreviations.—O. Reaction negative. + Reaction positive. ++ Reaction very strong.

which an albumen molecule is composed, are combined with one another.*

The method of separation cannot, however, be perfectly complete.

Classifications of the albumens have been partially based on the method of salting out, those albumens which are readily precipitated being placed in a different class to those which require a higher concentration of salt for separation from solution. This empirical method will, however, probably be abandoned, owing to the improvement in the methods of investigation of the chemistry of the albumens, which we shall proceed to discuss next time, and future classifications will then be based on purely chemical differences of the various classes.

The Albumens as Chemical Entities. Crystallised Albumens.

In the case of simple organic bodies, we have many criteria for determining whether a body is a chemical entity or not. We can ascertain, for example, the boiling-point, melting-point, crystalline form, etc. In the case of the albumens, our principal criterion is, we have seen, the degree of concentration of different salts necessary for the precipitation of the body from solution. But even this factor is insufficient to allow us to state whether any particular product is a chemical entity, or whether it is a mixture of several different compounds. Considerable interest, therefore, is attached to the discovery that several albumens can be obtained in definite crystalline form.

Many albumenous bodies have been observed in a crystalline form in nature. It is only necessary to mention here the so-called "aleurone" grains that have been observed in many plants, and the hæmoglobin crystals, so readily obtainable from blood. The development of the technique, however, for the *artificial* preparation of crystallised albumens led to a new

* The experimental method for the separation of albumenous bodies by fractional precipitation with salts is discussed in greater detail in Lecture VIII.

method for obtaining pure bodies in a class of compounds the chemical manipulation of which is excessively difficult.

Apart from the preparation of hæmoglobin crystals, which are obtained with readiness, great interest attaches to the preparation of crystals from other albumens, which for a long time have been known only in an amorphous state. When, therefore, Gürber (2) succeeded in obtaining serum albumen in a crystalline form, and Hofmeister (4) prepared a crystallised egg albumen, it was generally admitted that a great advance had been made towards an accurate study of the albumens. In addition to the researches just mentioned, we must refer to Osborne's (7) work on the preparation of a crystalline albumen from hemp-seed, to which he gave the name "edestine." Specially important is the discovery of this last-named body on account of the ease with which it can be prepared on a comparatively large scale, and readily utilised therefore, for the purposes of further research.

Later investigators have improved the methods of the early discoverers of these crystalline products, and it is not necessary to give the details here. It will be sufficient to quote the description of the preparation given by Hopkins (6), of the improved method discovered by himself and Pinkus for the preparation of crystallised egg albumen.

"The egg-white is beaten to a froth (to break up the membranes) with exactly its own bulk of saturated ammonium sulphate solution. The mixture having stood over-night, or at least for a few hours, is filtered from the precipitated proteid. The filtrate is now measured. Ten per cent. acetic acid (glacial acid diluted to ten times its bulk) is then very gradually added from a burette until a well-marked permanent precipitate first forms—a precipitate sufficient to make the mixture actually milky in appearance; not a mere opalescence, for which liberated gas-bubbles might be mistaken. The actual amount of acid required to produce such a precipitate will vary (chiefly because of the varying loss of ammonia which occurs when the mixture has previously stood in open vessels). The point corresponds roughly to an incipient acidity of the liquid towards

litmus; but the formation of the permanent precipitate forms itself the best indicator. This stage being reached, a measured quantity of the acid is now added, over and above that required to produce the first precipitate; 1 cubic centimetre extra being added for each 100 cubic centimetres of the filtered mixture as originally measured. The whole contains, therefore, approximately 1 part per mille of free acid. . . . The bulky precipitate thus produced is at first amorphous, and if the mixture be occasionally shaken, the amorphous precipitate will give place to crystals within four or five hours. To obtain the full yield, however, the material should stand for 24 hours." The product thus obtained by Hopkins is already nearly pure.

On recrystallising once more from ammonium sulphate (dissolving in water and then carefully adding half-saturated ammonium sulphate solution containing acetic acid in the proportion of 1 per mille till a permanent precipitate forms, and then about 2 c.c. ammonium sulphate solution per litre in excess of this) a perfectly pure preparation is obtained, which gives constant numbers on analysis after further recrystallisation, and also shows a constant specific rotatory power when the solutions are examined by the polariscope.

It has been stated before that it is extremely difficult, if not impossible, to remove an albumen from its position *in situ* in an organism, without alteration. The relation of a crystalline albumen prepared by a method like the one just described, to the albumen as it exists in the egg-white, is unknown. In view of the considerations already advanced with regard to the pseudo-acid and pseudo-base-like character of an albumen, it seems extremely unlikely that Hopkins' crystallised egg albumen is identical with the natural albumen of egg-white, especially when we remember that acetic acid plays a distinct rôle in the formation. It is conceivable, for example, that acetic acid converts the albumen into a basic form, and possibly combines with the body thus produced to form a salt. Nevertheless, the preparation of an albumen body with constant properties is a distinct advance in our technique, and even if the crystalline body be not identical with the natural product, it

can hardly differ from the latter, as far as its chemical construction is concerned, in any very appreciable degree.

REFERENCES—LECTURE I

- (1) BIAL.—*Zeitsch. f. klin. Medicin*, 50, p. 417, 1903.
- (2) GÜRBER.—*Trans. Physic. Med. Soc. of Würzburg*, p. 143, 1894.
- (3) HANTZSCH.—*Ber.*, 32, p. 575, 1899.
- (4) HOFMEISTER.—*H.Z.*, 14, p. 165, 1889.
- (5) HOPKINS and COLE.—*Proc. Roy. Soc.*, 68; and *Jour. Physiol.*, 27, p. 418, 1901.
- (6) HOPKINS and PINKUS.—*Jour. Physiol.*, 25, p. 306, 1900.
- (7) OSBORNE.—*Amer. Chem. Jour.*, 14, p. 208, 1892.
- (8) PICK.—*H.Z.*, 24, p. 246, 1897; *H.Z.*, 28, p. 219, 1899; and *Hofmeister's Beiträge*, 2, p. 481, 1902.
- (9) ZUNZ.—*H.Z.*, 28, p. 132, 1899; and *Hofmeister's Beiträge*, 2, p. 435, 1902.

LECTURE II

THE DEGRADATION PRODUCTS OF THE ALBUMENS

It has been shown that the albumens generally give with reagents certain definite reactions, which indicate in the albumen molecule the presence of various chemical groups. The biuret reaction, for example, indicates, as Schiff has shown, the presence of bodies derived from condensation of carboxyl- and amino- or amido-groups (see p. 8), the Molisch reaction the presence of carbohydrate groups, and the Millon reagent the presence of oxyphenyl groups.

It is of importance now, to elaborate a method by which these various groups may be isolated in a pure state and identified.

In order to determine the simpler groups contained in a molecule, various methods have been employed by chemists. The chemical processes generally adopted are destructional distillation, oxidation, and hydrolysis. The first of these methods is not generally applicable to bodies of high molecular weight like the albumens. The third method, viz., that of hydrolysis, is the one that has been most extensively adopted in the case of the albumens, as it can be applied under very varying conditions.

Hydrolysis has been performed by means of the following reagents :—

- (i) By superheated water vapour.
- (ii) By acids of varying concentration.
- (iii) By alkalis of varying concentration.

(iv) By means of enzymes, of which the principal are :—

(a) peptic enzymes (in acid solution).

(b) tryptic enzymes (in alkaline or neutral solutions).

(c) papayotine (the pine-apple ferment in acid or alkaline solution).

(d) autolytic enzymes.

(v) By fusion with alkalis. This last-named process is more violent, and is apt to be complicated by concurrent oxidation.

In addition to the purely chemical methods, the degradation of albumens has been studied in the living organism during the processes of normal metabolism. Another, and analogous method of observation, is afforded by the study of degradation caused by bacteria, such as takes place during the normal processes of putrefaction. This last method is of considerable importance, as, by this means, products different to those obtained in hydrolysis are produced; the two sets of products bear, nevertheless, a certain chemical relationship to one another. The production from the same albumen of different products has afforded considerable help, as will be seen later on, in the determination of the chemical constitution of the degradation products.

It was long ago observed that, in certain albumen bodies, in the earlier stages of degradation a scission takes place of definite groups, which are often of large molecular weight, and which do not possess the general character of albumens. These groups were termed by Hoppe-Seyler the *prosthetic groups*, and albumens containing complexes of this nature were called *proteids*, in contradistinction to the albumens ("Eiweiss stoffe"), in which prosthetic groups were absent. In English it has not been the custom to make this distinction, and the generic term *proteid* was applied to all albumens indiscriminately. It is convenient, however, to bring our English nomenclature into line with the German, and throughout these lectures the distinction between a proteid and an albumen will be maintained.

The following are some typical examples of proteids and their prosthetic groups :—

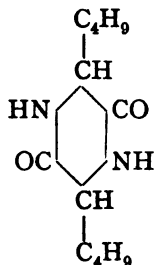
<i>Proteid.</i>	<i>Prosthetic group.</i>
Glyco-proteid.	Carbohydrate.
Nucleo-proteid.	Nucleic acid.
Hæmoglobin.	Chromatogenic group.

The degree of degradation varies with the reagent employed in hydrolysis. By means of the enzymes we can produce degradation products which are still quite complex, and which still retain to a great extent the general characters of the albumens. Such bodies are the albumoses and peptones. By means of more powerful hydrolysing reagents, such as mineral acids, acting at the temperature of boiling water, the simpler products are readily obtained. These products no longer show the characteristic reactions of the albumens. If, now, the simpler products be studied first, and identified or synthesised, the subsequent study of the more complex groups, such as the albumoses and peptones, is facilitated. The separation of such complex bodies, by methods like the one described in the last lecture (p. 10) and applied by Pick to the product known as Witte's peptone, and the identification of the groups comprised in these more complex products, will ultimately throw considerable light on the constitution of the albumens. This study has as yet not proceeded very far, as it is only within the last few years that the systematic method for the isolation and identification of the simple degradation products has been elaborated.

Nevertheless, during the course of many years work, a large number of degradation products from different albumens have been isolated and identified, although it is only quite recently that Emil Fischer and his pupils have employed a systematic method in this work.

It will be convenient, before proceeding to discuss the experimental methods of Fischer, to give an account of the simple degradation products that have been obtained from various albumens, and to indicate the syntheses by means of

belongs to a class of bodies known as the peptides. Leucinimide should not, properly speaking, be classified amongst the ultimate degradation products. It has the constitution



II. The monoamines of dibasic acids.

Aspartic acid - $\text{CH}(\text{NH}_2) \cdot \text{COOH}$

$|\text{CH}_2 \cdot \text{COOH}.$

Glutaminic acid - $\text{COOH} \cdot \text{CH}(\text{NH}_2) \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}.$

G. THE DIAMINO ACIDS.

I. The so-called hexone bases.*

Arginine. $|$ *Lysine.* $|$ *Histidine.*

II. Other diamino acids.

(?) *Diamino-glutaric acid* - $\text{C}_5\text{H}_{12}\text{O}_4\text{N}_2$ (Skraup).

(?) *Diamino-adipic acid* - $\text{C}_6\text{H}_{14}\text{O}_4\text{N}_2$ (Skraup).

H. HYDROXYAMINO ACIDS.

Serine - $\text{CH}_2(\text{OH}) \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}.$

Tyrosine.



$\text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}.$

(?) *Amino-hydroxy-succinic acid.* (Skraup).

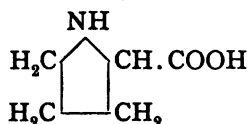
(?) *Diamino-dihydroxy-succinic acid.* (Skraup).

Diamino-trihydroxy-dodecanic acid - $\text{C}_{12}\text{H}_{26}\text{O}_6\text{N}_2.$

* This term is retained for convenience. These bodies will be considered in detail in the next lecture.

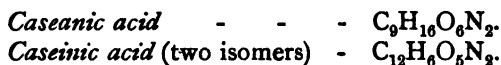
† The abbreviated formula is employed for the benzene ring.

I. PYRROLIDINE-CARBOXYLIC ACID, or *Proline*, isolated in recent years by E. Fischer,



and also its hydroxy-derivative (oxyproline).

J. CERTAIN ACIDS OF UNKNOWN CONSTITUTION, recently isolated by Skraup from the products of hydrolysis of casein, viz.,



K. HYDROXYPHENYLETHYLAMINE, PUTRESCINE AND CADAVERINE (Langstein) (13).

The above products are obtained principally by hydrolysis, but we must also mention here the important products obtained by putrefactive degradation, viz., various bases such as putrescine, cadaverine, etc., and the skatol and indol derivatives, such as skatolamino-carbonic and acetic acids.

We will now proceed to discuss the chemistry of these groups in detail.

GROUP A.

As to the rôle played by the simple volatile products in the molecule, we know at present very little; they are nearly always eliminated on hydrolysis, and may be due to decomposition of other degradation products, and therefore of secondary importance.

GROUP B.

Pyrotartaric acid has recently been isolated by Mörner (14) amongst the products of hydrolysis of horn and hair. It appears to be a secondary decomposition product, derived possibly from cystine.

GROUP C.

The Carbohydrate Groups.

It has been already mentioned that certain proteids, amongst

which are the mucines and cartilage, readily yield a carbohydrate group on hydrolysis. The carbohydrate complexes in these cases form the prosthetic groups, and the proteids belong to the class of glyco-proteids. These bodies will receive our consideration later. We are now concerned with the carbohydrate groups which are intimately bound up in the molecule of an ordinary albumen, such as serum-albumen and egg-albumen. The question as to whether the molecules of these bodies contain normally these groups, is one of great physiological importance, and is intimately connected with the general pathology of diabetes. It is not surprising, therefore, that a large literature, much unfortunately of controversial and often contradictory nature, is centred round this subject.

We have already considered the various qualitative reactions which indicate the presence of a carbohydrate group in the albumen molecule (the reactions of Molisch, Bial, etc.). Attention has also been called to the fact that Pick, in his examination of the products of peptic digestion of the albumens, has shown that certain fractions of Witte's peptones give the Molisch reaction very strongly, whereas in other fractions the reaction is very weak. The former fractions for this reason are called the glykalbumoses.

Glykalbumoses have also been obtained from the liver, which are analogous in their properties to the albumoses prepared by artificial digestion. Simon (21) succeeded by means of fractional precipitation with alcohol, in isolating such a body, and preparing therefrom by hydrolysis a substance yielding an osazone. Seegen (20), in his last work, carried out in conjunction with Neimann, also isolated from the liver a nitrogenous product, which, on treatment with 2 per cent. hydrochloric acid in a sealed tube, yielded a strongly reducing body with properties very similar to grape-sugar.

We must now consider the attempts that have been made to prepare directly a carbohydrate from such albumens as those of serum and egg.

In the case of egg-albumen considerable controversy has occurred, owing to the fact that eggs contain a true glyco-

protein, and the question has continually arisen as to whether the product used by the different investigators in their researches has been entirely free from this substance.

The first investigator who demonstrated the presence of a carbohydrate group in egg-albumen was Schützenberger. He hydrolysed coagulated and well-washed egg-white with barium hydrate at 100° C. for 120 hours. Ammoniacal lead salts precipitated a body which was insoluble in alcohol, which, although not itself a reducing body, yielded such a substance on further hydrolysis with sulphuric acid.

Pavy has also carried out extensive researches (18) on this subject. He hydrolysed egg-white with 10 per cent. caustic soda solution, and obtained, after neutralisation with acetic acid, and precipitation with alcohol, a body which also on further hydrolysis with acids yielded a reducing product, from which an osazone could be prepared.

Our most recent knowledge on the subject of the carbohydrates is due, however, to the work of Friedrich Müller and his pupils, especially Seemann (15) and Fränkel (8) (whose work was directed towards the isolation of the parent substance, *i.e.*, a starch or gum-like body from which the reducing body is formed by hydrolysis), of Blumenthal and Meyer (2), and of Langstein (31).

It is not possible to consider in detail all these researches, but all tend to show that carbohydrate groups form no inconsiderable part of the whole albumen molecule. Seemann succeeded in isolating sugar from the degradation products by means of the following method. He hydrolysed a purified egg-albumen with 3 per cent. hydrochloric acid, and treated the products thus obtained with benzoyl chloride by the Baumann-Schotten method (*i.e.*, in the presence of an alkali). By this means benzoyl derivatives are obtained, which can be readily purified by recrystallisation. On hydrolysis of the purified benzoyl sugar obtained in this way, the pure sugar was prepared. This was found to be identical with *chitosamine*, or *glycosamine*, a nitrogenous carbohydrate.

These results were confirmed by Langstein, who succeeded

in obtaining from crystallised egg-albumen as much as 10-11 per cent. of a body capable of reducing Fehling's solution.

The presence of chitosamine in albumens has also been confirmed by Neuberg (16), who employed another experimental method. He hydrolysed egg-albumen with hydrobromic acid, and oxidised the products of hydrolysis with bromine water. He then fractionally precipitated the oxidation products with lead acetate, and obtained a lead salt, which was decomposed with sulphuretted hydrogen. A crude acid was thereby obtained, which was neutralised by quinine or cinchonine. These bases form with the acid beautiful crystalline salts, which can be readily purified by recrystallisation. By these means an acid, *norisosaccharic acid*, was obtained in a pure state, a body which had been previously obtained as an oxidation product of chitosamine by Fischer and Tiemann.

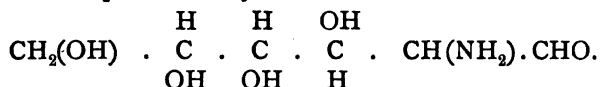
From these researches, there can be little doubt that chitosamine is obtained in not inconsiderable quantities by the degradation of egg-albumen. It possibly exists in the molecule in the form of a polysaccharide (the "albamin" of Fränkel), but so far no one has succeeded in obtaining such a body in a pure state.

Glycosamine is obtainable from other albumens, and its presence in serum-albumen has been recently demonstrated by Langstein.

Constitution of Glucosamine or Chitosamine.

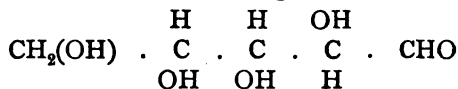
The constitution of this body is now known, from the synthesis of Fischer and Leuchs (6).

It can be represented by the formula



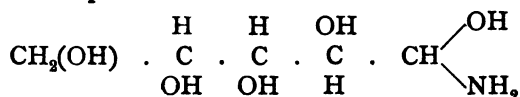
It was synthesised by the following method:—

Arabinose, a five-carbon chain sugar, of known constitution, viz.,

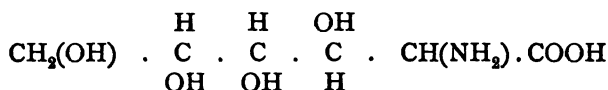


was treated with ammonia.

The addition product thus formed



was then treated with hydrocyanic acid. The hydroxyl group was thereby replaced by the (CN) group, which was subsequently readily hydrolysed to the carboxyl (COOH) group by the ordinary reaction. By this means α -glucosaminic acid,



is formed. This product can also be obtained from glucosamine directly by oxidation, and this fact alone would be sufficient to establish the constitution of the sugar. Fischer and Leuchs, however, succeeded in directly synthesising this latter body. They treated the glucosaminic acid with alcohol and hydrochloric acid, and obtained in a crude state a syrupy body, which is probably a lactone of the acid. This on reduction with sodium amalgam yielded glucosamine, which was isolated in the form of a crystalline derivative.

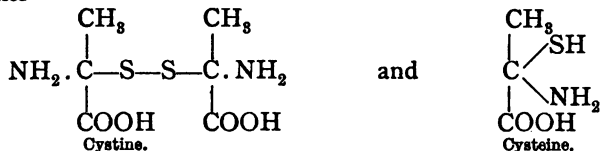
GROUP D.

The Sulphur Bodies.

It has been already shown that albumens on treatment with lead salts in the presence of caustic alkalis yield a black colouration, indicating the presence of sulphur in the molecule. It has been observed, however, that the whole of the sulphur cannot be eliminated by this reaction, and it was formerly believed that two classes of sulphur groups were present in the molecule, viz., those in which the sulphur was eliminated by the "lead sulphide" reaction, and those which were resistant to the reagents used in this test. This subject has recently been investigated in detail by Mörner (14a), to whom we are indebted for a method for the isolation of the sulphur degradation products. The only product known with certainty, viz., cystine,

was shown by Mörner to yield only part of its sulphur when treated under certain definite conditions with alkali and lead salts. It was also shown that from bodies containing relatively large quantities of cystine, such as human hair or horn, approximately the same percentage of the total sulphur was eliminated under the same conditions, a fact which led Mörner to conclude that a division of sulphur bodies into two groups was not justifiable. So far as we know, then, cystine is the principal sulphur-containing body obtainable by albumen degradation. This body is of considerable physiological interest, as it occurs in small quantities in normal urine, but under certain conditions, *i.e.*, in cystinuria, it is found in relatively large quantities and forms the main constituent of the urinary calculi found in these cases. It was originally discovered by Wollaston in 1810.

Its empirical formula was first thoroughly established by Baumann (1) in 1884, who found that it readily yields on reduction another base, *viz.*, cysteine, $C_3H_7NSO_2$. The relation of cystine to cysteine, Baumann found to be that of a mercaptan to a disulphide. For the two bodies Baumann suggested the formulæ



Later researches tend to show, however, that these formulæ are probably incorrect.

The two investigators to whom we owe our most recent knowledge on this subject are Mörner (*loc. cit.*) and Friedmann (9). Mörner, to whose work reference has been already made, systematically investigated the sulphur group of a large number of albumens, from which he succeeded in isolating cystine in a pure state. It can be obtained from serum-albumen, egg-albumen, and several other substances, but the best sources are horn and human hair. From the latter, yields up to about 11 per cent. of the dried substance can be obtained; hence, there is no difficulty now in obtaining supplies

of material for investigation of the constitution, and chemists have not to rely upon urinary calculi from cases of cystinuria as their sole source of supply of this body. The following is a description of Mörner's procedure for preparing cystine from hair; a similar method is applicable to its preparation from other albumens.

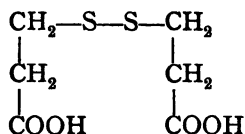
150 grams of hair, previously dried at 120° C. are heated with 350 c.c. hydrochloric acid (sp. gr. 1.124) and 300 c.c. water, for seven days on a water-bath. The temperature of the mixture is kept at about 93°. The solution is then decolourised with animal charcoal, and concentrated to a small bulk in vacuum. The residue is then taken up with 350 c.c. water, and 650 c.c. alcohol are added. This solution is then neutralised with caustic soda in 50 per cent. alcohol, and made slightly acid with acetic acid. After standing for two days, the precipitate which has formed is filtered off. In the case of human hair this precipitate consists principally of cystine, but in other cases a mixture of tyrosine and cystine is thus prepared. These two bodies can be separated by fractional crystallisation from dilute ammonia. For this purpose, the crystals are dissolved in ammonia, and the solution thus formed is decolourised with animal charcoal, and concentrated to crystallisation point under diminished pressure. The cystine obtainable by this method amounts to about 11 per cent. of the weight of dried hair.

Mörner found that the cystine has a tendency to be converted into another modification, which is less lævo-rotatory, and which is probably a stereo-isomer.

The researches of Friedmann deal with the constitution of the body.

He applied to cystine a reaction discovered by Jochem in Hofmeister's laboratory, which consists of treating an amino-acid dissolved in concentrated hydrochloric acid with sodium nitrite. By this means the amino-group is replaced by chlorine, and a chloro-acid is thereby formed. In the case of cystine, a dichloro-dithiolactic acid is formed, which, on reduction with zinc and hydrochloric acid, yields a thiolactic acid. This body

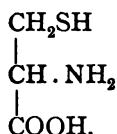
on treatment with ferric chloride is oxidised to a dithio-acid. The body is identical with the acid—



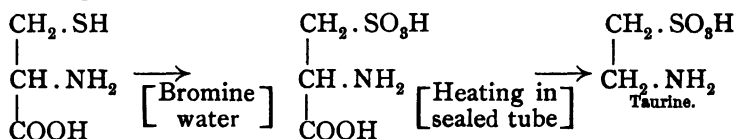
which can be obtained synthetically.

This indicates that the thio-group is in the β position, and not in the α position, as supposed by Baumann.

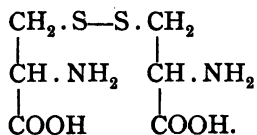
Friedmann also discovered another series of reactions. He found that, on oxidation of cysteine with bromine water, an acid is obtained which, on heating with water in a sealed tube, is converted into taurine with evolution of carbonic acid gas. This body has a known constitution, and the sulphur-containing and amino-groups are on different carbon atoms. This suggested for cysteine the following formula—



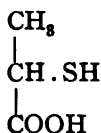
Its conversion into taurine would then be represented by the following formulæ—



This preparation of taurine from cystine is of physiological interest, in that it suggests the origin of the former body in the bile. It would appear from these researches that the formula of cystine is the following—

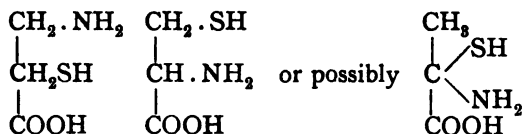


In spite of the interesting results obtained by Friedmann, the constitution of cystine can by no means be regarded as definitely established. Several observers have obtained α thiolactic acid



amongst the products of hydrolysis of cystine. Mörner, in his latest paper on the subject has confirmed this result, for he obtained in addition to the β acid, on heating the hydrochloride of cysteine to 140° with water, a minute quantity of α -thiolactic acid, together with sulphuretted hydrogen, ammonia, and alanine— $\text{CH}_3 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$.

It is not easy to draw a very definite conclusion from these results, as the presence of alanine tends to confirm the formula of Friedmann. It must be noted, however, that the yields of taurine and other products are by no means quantitative. Furthermore, the whole of the sulphur in pure cystine cannot be eliminated by means of the alkali-lead salt method. It is, therefore, possible that one or more of the following forms of cysteine, viz.,



form the basis of the cystine molecule, and that they are eliminated by hydrolysis, either in the form in which they pre-exist in cystine, or can be converted one into another by isomeric changes, during their elimination by the process of hydrolysis. It is not yet certain, either, whether the cystines prepared directly from albumens by Mörner's method and the cystine from urinary calculi are identical. This question is being investigated by Neuberg and Mayer. Erlenmeyer has also been engaged in attempting the synthesis of cystine, and until various researches now in progress are completed, we

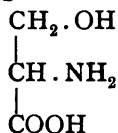
cannot assign any definite formula to this interesting and physiologically important body.*

GROUP H.

Serine.

This body, which was originally obtained from silk-fibroine, and has since its discovery been repeatedly isolated from the degradation products of albumens in the form of its ethyl ester by Fischer's method (to be described in the next lecture), is in reality a hydroxy-amino acid. Owing to its chemical relationship to cystine, it can be conveniently considered in this place.

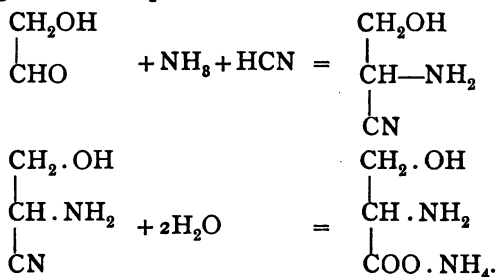
It possesses the following constitution—



i.e., it is analogous to cysteine, having a hydroxyl group in the molecule instead of a mercaptan group.

It has been synthetically prepared by Fischer and Leuchs (7) by the following method.

Glycolyl aldehyde is treated with ammonia and hydrocyanic acid simultaneously, and the amino-nitrile thus prepared is directly hydrolysed, without being isolated in a pure state. The following formulæ represent the reactions—



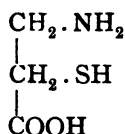
The yield obtained in this synthesis is small. The constitution of serine may be regarded, however, as definitely proved.

* See, however, addendum, p. 33.

ADDENDUM.

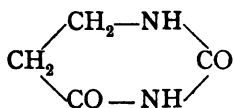
Since the preparation of this lecture, some important researches bearing on the constitution of cystine and serine have been published.

Gabriel (19) has succeeded in preparing a body of the constitution

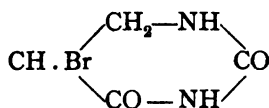


which is isomeric, but not identical with cysteine. He calls the body isocysteine, and he obtained it by the following series of reactions :—

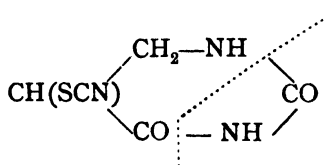
β -Lactyl urea,



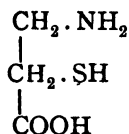
on treatment with bromine, yields a monobrom-derivative,



which on treatment with potassium thiocyanate gives the body



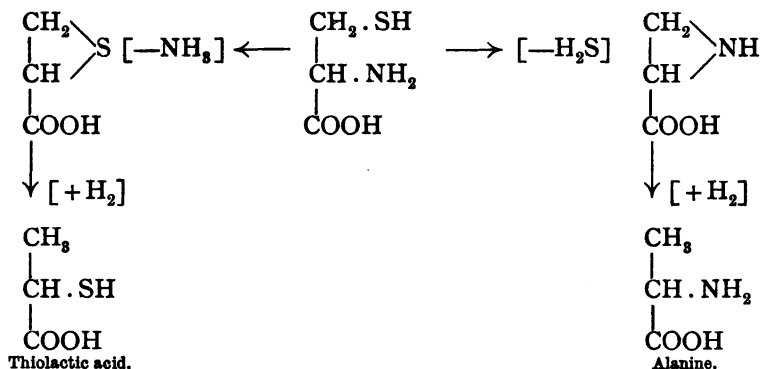
which on hydrolysis with hydrochloric acid at a high temperature yields



C

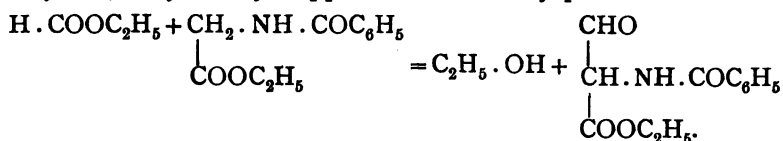
a scission of the molecule taking place at the points indicated by the dotted line.

Gabriel explains the formation of α -thiolactic acid and alanine from cystine, by assuming that scission of either ammonia or sulphuretted hydrogen from cystine can occur, and that the intermediary bodies thus produced can be readily reduced by hydrogen. The following scheme explains Mörner's results:—

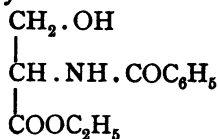


Erlenmeyer (5) has recently succeeded in synthesising both serine and cysteine by similar methods, and thus establishing the close chemical relationship of these bodies. The following methods were employed.

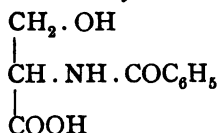
Ethyl formate and ethyl hippurate (the ethyl esters of formic and hippuric acids) were condensed by means of sodium ethylate; ethyl-formyl-hippurate was thereby produced.



This body, on reduction with aluminium amalgam in ethereal solution, yields the body

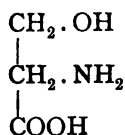


which on hydrolysis with alkalis yields



i.e., benzoyl-serine.

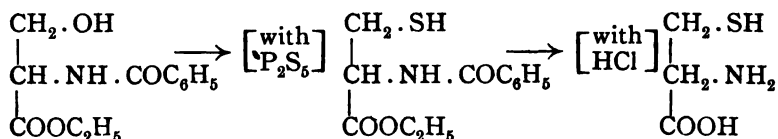
Benzoyl-serine on hydrolysis with dilute sulphuric acid yields serine



which can by this method be produced on a comparatively large scale.

Cysteine can be produced from the reduction product of ethyl-formyl-hippurate, already mentioned, by the following reactions. On treatment with phosphorus pentasulphide a thio-derivative is produced, which on hydrolysis with acids yields cysteine.

The formulæ can be represented as follows—



The synthetic cysteine on oxidation with air in ammoniacal solution is readily converted into cystine. The synthetical products are, of course, optically inactive. Question has arisen as to whether the cystine obtained by the hydrolysis is identical or not with that obtained from urine and from calculi. Neuberg and Mayer (17) maintain that the calculus cystine differs from the proteid or urinary cystine, but that the products from the two latter sources are identical. Rothera (19), on the other hand, maintains that the calculus cystine is identical with that obtained by the hydrolysis of albumens.

For details as to this controversy, reference must be made to the original papers (pp. 17 and 19).

GROUP E.

Tryptophane.

Tryptophane is the product yielding the colour reaction originally discovered by Adamkiewicz and modified by Hopkins and Cole (11). The actual isolation of this group is due to the researches of these two latter investigators. It is of considerable physiological interest, on account of its intimate chemical relationship to other bodies isolated from the products of putrefactive degradation of albumens.

The researches which culminated in the isolation of tryptophane were undertaken by Hopkins and Cole with the express purpose of isolating the tryptophane-yielding body. The following method was elaborated.

As a source, a commercial casein preparation was employed. 1 kilo was placed in 10 litres of 0.8 per cent. carbonate of soda solution, and digested at 35° C. with 400 c.c. of Benger's liquor pancreaticus, which was added in two portions, half at the outset and the other half at the end of the first or second day of digestion. 1 part per mille sodium fluoride and 50 c.c. chloroform were added, to keep the mixture aseptic. The digestion was continued until the tryptophane reaction had reached its maximum of intensity, which generally happened after five to seven days. The mixture was then heated to 80° C., and after cooling filtered. Sulphuric acid was added to the filtrate in such quantities as to equal about 5 to 6 per cent. of the volume. If calcium was present in the products of digestion, the mixture was allowed to stand for some hours, to allow of the separation of calcium sulphate. After filtration, a special reagent of Hopkins and Cole was added, viz., mercuric sulphate, dissolved in 5 per cent. sulphuric acid. This solution should contain about 10 per cent. of the mercury salt. This reagent was added in sufficient quantity, and after this mixture had stood for some time, a bulky lemon-yellow precipitate formed. This was then filtered off and washed thoroughly with 5 to 6 per cent. sulphuric acid. It was then treated with

sulphuretted hydrogen, and the filtrate from the mercuric sulphide was freed in the usual way from excess of the gas. Sulphuric acid was then added again, in such quantity that it was equal to about 5 to 6 per cent. by volume of the liquid, and then the mercuric sulphate reagent was added, and the tryptophane body reprecipitated. In the second precipitation cystine can be almost completely separated by adding only just enough of the mercury reagent to produce a permanent precipitate, allowing to stand for half an hour after thoroughly shaking, and rapidly filtering. Almost the whole of the cystine is carried down in this first-formed precipitate.

On adding the mercuric sulphate reagent to the filtrate, the tryptophane compound was reprecipitated. The precipitated mercury salt was decomposed by sulphuretted hydrogen in the usual way, the sulphuric acid in the filtrate from the mercury sulphide was precipitated quantitatively as a barium salt, and the filtrate from the barium sulphate evaporated down with continual additions of alcohol. A crystalline body then separated out, which on recrystallisation from aqueous alcohol, after decolourisation with animal charcoal, was obtained in a state sufficiently pure for analysis.

Properties and constitution of Tryptophane.—The crystalline body thus obtained gives on distillation an abundant yield of indol and skatol, and this immediately suggested the relationship of this body to various products obtained from the putrefaction of albumens. Amongst such products the following have been described.

Indol. (Kühne, and also Nencki.)

Skatol. (Brieger.)

The so-called skatol-carbonic acid. (E. and H. Salkowski.)

The so-called skatol-acetic acid. (Nencki.)

Now it has been established that from tyrosine, *p*. hydroxyphenylacetic and *p*. hydroxyphenylpropionic acids are obtained by putrefaction.

The chemical relationship can be represented by the following formulæ :—



$\text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$
Tyrosine.



$\text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$
p. Hydroxyphenyl-
propionic acid.

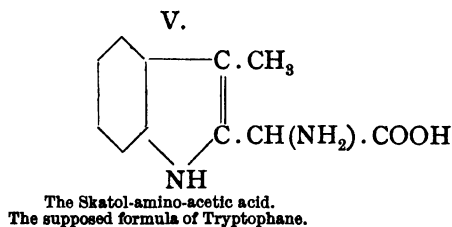
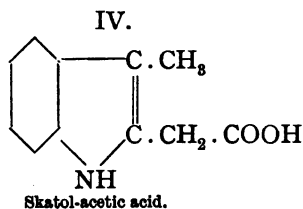
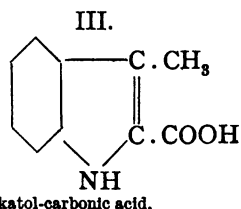
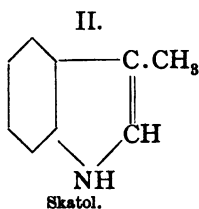
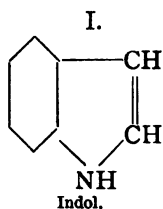


$\text{CH}_2 \cdot \text{COOH}$
p. Hydroxyphenyl-
acetic acid.

Phenol and cresol can also be obtained.

As these products are obtainable from tyrosine by putrefaction, Nencki and Salkowski assumed that indol, skatol, and the so-called skatol-carbonic and skatol-acetic acids are derivable from an amino-derivative, which Nencki supposed to be skatol-amino-acetic acid. This latter body, the existence of which was predicted from the discovery of the various indol and skatol bodies prepared from the products of putrefaction, has the same empirical formula as the tryptophane body isolated by Hopkins and Cole. The latter assumed, then, that this body was the skatol-amino-acetic acid, of which the existence had been predicted.

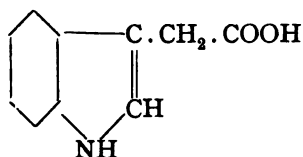
The chemical relationship of these bodies, as originally suggested by Nencki, would be represented by the following formulæ :—



* The abbreviated benzene formula is used throughout.

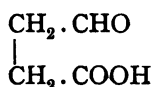
Although the surmise as to the existence of a body of the empirical formula of skatol-amino-acetic acid has proved correct, the actual constitutional formulæ for the various bodies proposed by Nencki will have to be modified, as Ellinger (4) has shown in some recently published researches.

Ellinger draws attention to the fact that a skatol-carbonic acid of the formula III. has been actually synthesised by Wislicenus and Arnold (23), and that it is *not* identical with the acid of the same empirical formula isolated from the products of putrefaction of albumens. He shows that the latter acid has the formula

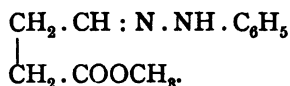


i.e., it is an *indol-acetic acid*, and *not a skatol-carbonic acid*, as Nencki thought.

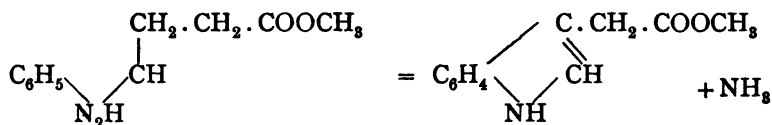
He obtained it synthetically in the following way. He prepared the semi-aldehyde of succinic acid, viz.,



and condensed the methylic ester of this body with phenylhydrazine. He then obtained the product



This on heating with alcoholic sulphuric acid undergoes a transformation represented by the following equation—

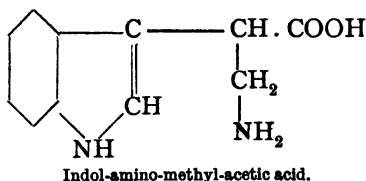


and the body thus obtained yields on hydrolysis indol-acetic

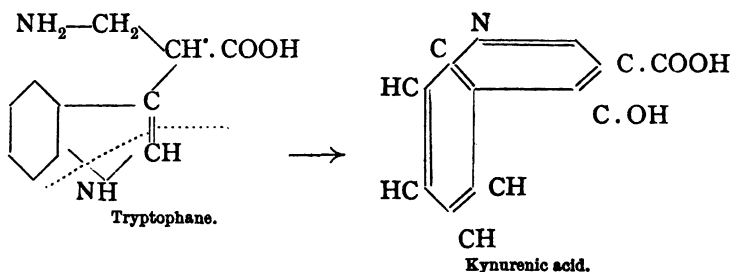
acid, which is identical in properties with the body of the same empirical formula obtained from the products of putrefaction.

This somewhat complex synthesis, taken in conjunction with the synthesis of Wislicenus and Arnold of the isomeric body, proves fairly conclusively the constitution of the so-called skatol-carbonic acid of putrefaction. This name must, however, now be dropped, and the acid called indol-propionic acid.

In view of these results we must adopt another formula to represent tryptophane. Ellinger shows that, on the assumption that it bears to the indol and skatol bodies of putrefaction the same chemical relationship that tyrosine bears to hydroxy-phenyl-propionic and acetic acids, phenol and cresol, four formulæ are possible, but he chooses one as the most likely, viz.,



This formula Ellinger adopts on the ground that tryptophane, when administered to a dog, is secreted as an acid, viz., kynurenic acid, of which the constitution is known. This body is γ hydroxy- β quinoline-carbonic acid, and its production from tryptophane can be represented by the following formulæ :—



Furthermore, Hopkins and Cole obtained from tryptophane, by oxidation with ferric chloride, a product, C_9H_7NO , which is probably, Ellinger thinks, a hitherto unknown hydroxy-chinoline.

There is little doubt, however, that the hitherto so-called skatol-carbonic acid of putrefaction is in reality indol-acetic acid, and this fact must be taken into account in assigning a formula to the tryptophane body. The formula assigned to tryptophane by Ellinger cannot yet be regarded however, as definitely established.*

REFERENCES—LECTURE II

- (1) BAUMANN.—*H.Z.*, 8, p. 299, 1884.
- (2) BLUMENTHAL and MAYER.—*Ber.*, 32, p. 274, 1899.
- (3) EHRLICH (FELIX).—*Ber.*, 37, p. 1809, 1904.
- (4) ELLINGER.—*Ber.*, 37, p. 1801, 1904.
- (5) ERLENMAYER.—*Liebig's Annalen*, 337, p. 236, 1904.
- (6) FISCHER and LEUCHS.—(Glycosamine), *Ber.*, 36, p. 24, 1903.
- (7) FISCHER and LEUCHS.—(Serine), *Ber.*, 35, p. 3787, 1902.
- (8) FRÄNKEL.—A series of papers published in the course of the last few years by the Vienna Academy of Sciences.
- (9) FRIEDMANN.—*Hofmeister's Beiträge*, 2, p. 433, 1902.
- (10) GABRIEL.—*Ber.*, 38, p. 630, 1905.
- (11) HOPKINS and COLE.—*Jour. of Physiol.*, 27, p. 418, 1901.
- (12) LANGSTEIN.—(Carbohydrates), *H.Z.*, 31, p. 49, 1900; and, *H.Z.*, 42, p. 171, 1904.
- (13) LANGSTEIN.—(Products of Tryptic Digestion), *Hofmeister's Beiträge*, 1, p. 507, 1901; and, 2, p. 229, 1902.
- (14) MÖRNER.—(Pyrotartaric Acid), *H.Z.*, 42, p. 121, 1904.
- (14a) MÖRNER.—(Cystine), *H.Z.*, 28, p. 599, 1899; 34, p. 313, 1902; and, 42, p. 347, 1904.
- (15) MÜLLER and SEEMANN.—*Deutsche med. Woch.*, p. 13, 1899.
- (16) NEUBERG.—*Ber.*, 34, p. 15, p. 3480, and p. 3963, 1901; also, *Ber.*, 35, p. 8 and p. 1467, 1902.
- (17) NEUBERG and MAYER.—*H.Z.*, 44, p. 472, 1905.
- (18) PAVY.—*The Physiology of the Carbohydrates*, London, 1894.
- (19) ROTHERA.—*Jour. of Physiol.*, 32, p. 175, 1905.
- (20) SEEGEN and NEIMANN.—Vienna Academy, May 1903.
- (21) SIMON.—*A.P.P.*, 49, p. 6, 1903.
- (22) SKRAUP.—*H.Z.*, 42, p. 274, 1904.
- (23) WISLICENUS and ARNOLD.—*Liebig's Annalen*, 246, p. 334, 1886.

* See, however, Appendix.

LECTURE III

THE DEGRADATION PRODUCTS OF THE ALBUMENS—*continued*

GROUP F.

The Amino and Diamino Acids.

Fischer's Method of Isolation.—Various amino acids have been isolated by different observers from amongst the hydrolytic degradation products of albumens, and attempts have been made to estimate quantitatively the amounts formed. In view, however, of the recent advances in the technique for dealing with these bodies, which we owe to the researches of Emil Fischer and his pupils, it will be unnecessary to discuss in detail the earlier researches.

The main principal involved in E. Fischer's method is the conversion of the acids into their ethyl esters, and the partial separation of these bodies by fractional distillation under very diminished pressure. The method may be best illustrated by giving in some detail a concrete example of an albumen hydrolysis. For this purpose the following account is taken from one of the first of Fischer's (4) papers on this subject, viz., that dealing with the hydrolysis of caseine.

500 grams of caseine are mixed with $1\frac{1}{2}$ litres of hydrochloric acid (sp. gr. 1.19), and the mixture is repeatedly shaken for one hour and a half.

At the end of this period it will be found that the greater part of the albumen, has dissolved giving a solution of a dirty violet colour. The liquid is then heated with a reflux condenser

on a sand-bath for about six hours. The liquid is then evaporated a little, and then saturated with gaseous hydrochloric acid. After standing for three days in an ice-chest, a copious separation of crystals takes place. The liquid is then mixed with an equal volume of ice-cold alcohol, and the crystals are filtered off on a pump. These, on dissolving in water, and decolourising the solution with animal charcoal, are precipitated in a pure state by the addition of gaseous hydrochloric acid. They were found to be crystals of the hydrochloride of glutamic acid, and were obtained in a yield of about 10 per cent. calculated on the quantity of dry caseine employed.

The amino-acids in the mother liquor from glutamic (or glutaminic) acid are separated as esters, and the following process was employed. The filtrate from the glutamic acid was evaporated under diminished pressure on a water-bath. (The temperature should not exceed 40°C .) A dark-brown syrupy residue was obtained, which was then dissolved in $1\frac{1}{2}$ litres of absolute alcohol. Hydrochloric acid gas was then passed into the solution till the saturation-point was reached, and the mixture was then warmed for a short time on a water-bath. In order to completely esterify the acids, the alcohol was distilled off under diminished pressure, and the residue again dissolved in a fresh quantity of alcohol, and the mixture treated with hydrochloric acid and evaporated as before. If necessary, this process is repeated once or twice more.

A thick syrup is thus obtained, which contains the esters of the various amino-acids in the form of their hydrochlorides, and the next process consists in the preparation of the free esters from the hydrochloric acid salts. Owing to the ease with which these esters are hydrolysed by alkalis, this process must be carried out with very great care, and for this reason the experimental details given by E. Fischer are of great importance, as the value of the method depends to a very great extent on the immense improvements on the technique employed, when compared with the technique of the earlier observers.

For convenience of manipulation, the syrup obtained from 500 gr. of caseine is divided into four portions, each one of

which is manipulated separately. To the thick syrup, in a distillation flask, about half a volume of water is added; and the solution is then *cooled in a freezing-mixture*. Then very strong caustic soda is added until the free acid is approximately neutralised. A saturated solution of potassium carbonate is then added, and a fairly large quantity of ether. This first operation has for its object the separation of the esters of aspartic and glutamic acids, which are specially sensitive to the action of alkalis. The ether, after thoroughly shaking with the mixture, is poured off, and the liquid again cooled in a freezing-mixture. A fresh quantity of ether is then added, and then in small quantities at a time 33 per cent. caustic soda, together with *solid* potassium carbonate. After each addition the mixture is thoroughly shaken in order to bring the alkali into intimate contact with the pasty mass, and to bring the free esters into ethereal solution. It is advantageous to renew the ether several times, and the quantity of alkali added must be at least sufficient to combine with all the hydrochloric acid. Potassium carbonate must be added in such quantities that it forms a paste with the aqueous solution. The object of this is to facilitate the extraction of the esters, which are so readily soluble in pure water, especially those of the lower amino acids, such as leucine and alanine.

The combined ethereal solutions can then be dried, first by shaking for five minutes with potassium carbonate, and afterwards by standing for twelve hours over fused sulphate of soda. Other drying reagents, such as quick-lime, barium oxide, etc., are unsuitable, as they tend to decompose the ester. Even potassium carbonate, after long standing, is not without action on these unstable bodies.

After evaporation of the ether a residue containing the free esters is obtained, which in E. Fischer's earlier experiments was fractionated under a pressure of 8-15 mm. The above is an outline of the general method for a rough separation of the amino-acids. E. Fischer, in his first research on caseine, obtained after two fractionations the following products from 1 kilo of material—

1.	Fraction of B.P	40° to 55°	.	14	grams
2.	" "	55° " 65°	.	14	"
3.	" "	65° " 80°	.	25	"
4.	" "	80° " 85°	.	165	"
5.	" "	85° " 110°	.	18	"
6.	" "	110° " 120°	.	40	"
7.	" "	120° " 130°	.	28	"
8.	" "	130° " 160°	.	8	"
Total				.	<u>312</u> grams

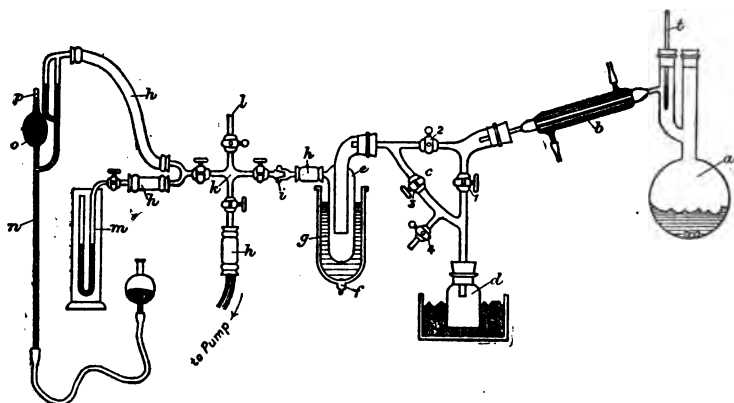
Now these fractions of esters do not contain pure products, but are themselves mixtures, and before considering the further separation, it will be necessary to call attention to the improvement of several details in the methods.

In the first place, Abderhalden has shown that by no means all the ester is extracted by the ether in the process above described. If the pasty residue after extraction with ether be made acid with hydrochloric acid, the salt which separates filtered off, and the filtrate evaporated to dryness and treated again with alcohol and hydrochloric acid two or three times, according to Fischer's method, a further not inconsiderable quantity of the hydrochlorides of the esters is obtained. Even the residue from these, after treatment with alkalis and ether, still contains unextracted esters. In order to obtain as nearly quantitative yields of the esters as possible, Abderhalden has shown that the additional manipulation as suggested by him must be repeated at least twice.

A further improvement, which has resulted in better yields and better fractionation, consists in the employment of very low pressures. Owing to the evolution of sulphuretted hydrogen and other gases during the distillation, it was a matter of considerable difficulty to operate at very low pressures (*e.g.*, at 0.2 mm.). To obviate the inconvenience caused by the evolution of gases during distillation, Fischer and Harries (3) devised an apparatus which is illustrated by the accompanying diagram.

The distillation flask (*a*) is heated in an oil-bath, the temperature of which is measured by a thermometer. The

particular form of flask employed, with extra side tube, is useful in cases where the liquid to be distilled is apt to foam over. The condenser (*b*) is cooled, in the case of distillation of high-boiling substances, with water, and in the case of low-boiling bodies with a well-cooled calcium chloride solution. This leads to an apparatus with four taps, by means of which the receiver (*d*) can be changed from time to time without interruption of the vacuum. This can be done by closing taps 1 and 3, and opening taps 2 and 4. By this means the receiver (*d*) is connected with the outer air, whilst the distillation flask



still remains connected with the vacuum pump. It can then be removed and replaced by a new receiver, when it is necessary to collect a new fraction. On connecting a new receiver, tap 2 is closed, and taps 1 and 3 are opened, and by this means a vacuum is rapidly produced in the receiver, and an additional fraction can then be collected.

Between the apparatus for collecting fractions and the pumps and manometers, is placed a condenser (*e*) standing in a "Dewar flask" (*f*), which is filled with liquid air (*g*). By this means all the volatile gases are condensed. The glass apparatus (*k*) has four taps, and serves to connect the distillation apparatus with the pumps, or with the pressure-measuring apparatus *m* and *n*. Through the fourth tap (*l*), air can be admitted into the system ;

m is an ordinary manometer. The other pressure-measuring apparatus is a modified M'Leod gauge, for determining pressures under 1 mm. For maintaining a vacuum, a Fleuss pump is employed.

The treatment of the various fractions of amino-esters varies with the hydrolysis products of different albumens. They are generally directly hydrolysed, the lower fractions by boiling for some hours with water in an apparatus connected with a reflux condenser, the higher fractions by boiling with baryta. The acids obtained by the hydrolysis can be further separated by fractional crystallisation.

*Some Remarks on the different Amino-Acids, and their
Methods of Isolation.*

Glycocoll.—This body can be readily separated, when it occurs amongst the products of hydrolysis in any considerable quantity, by taking advantage of the fact that the hydrochloride of the ethyl ester is only slightly soluble in alcohol. Thus, if the syrupy liquid obtained by the hydrolysis of gelatine be dissolved in alcohol and treated with hydrochloric acid, and the mixture be allowed to stand in a cool place for forty-eight hours, a large deposit of crystals of glycocoll-ethyl-ester hydrochloride is obtained. These can be filtered off, and the filtrate evaporated. The residue on the second treatment with alcohol and hydrochloric acid deposits a second crop of crystals. In this way Fischer, Levene, and Aders succeeded in separating nearly all the glycocoll from the hydrolysis products of gelatine.

Leucine and Alanine.—These bodies, obtained from the lower fractions of esters, can be separated from one another by fractional crystallisation. In the case of the hydrolysis products of caseine, they are probably mixed with a certain amount of amino-valerianic acid and other amino-bodies. Since Fischer carried out his experiments, F. Ehrlich has succeeded in showing that leucine, as ordinarily isolated from the products of hydrolysis, is a mixture of isomers, which can be separated by taking advantage of the fact that the copper salt of one isomer

is soluble in methyl alcohol, whereas that of the other is insoluble. In view of this discovery, the leucine containing fractions of the amino-acid esters requires re-examination. The addition products formed with phenyl-isocyanate are useful for identifying the products of this fraction.

Pyrrolidine-carboxylic Acid.—This acid was originally isolated by E. Fischer from amongst the hydrolysis products of caseine. It occurs chiefly in the ester fractions of intermediary boiling-point. It is readily soluble in water, and can be separated from the other acids of the fraction in which it is found by taking advantage of the fact that it is readily soluble in alcohol. This acid is isolated as a mixture of isomers, viz., a lævo-rotatory variety, and an inactive variety which has been racemised during the process of hydrolysis. The two forms can be separated by conversion of the acids into their copper salts, and extraction of the former with alcohol. The active variety yields a soluble, whereas the racemic form gives an insoluble copper salt.

A question has been raised as to whether pyrrolidine-carboxylic acid is a primary or secondary product of hydrolysis, formed in the latter eventuality from a diamino-acid. E. Fischer is of the opinion that it is a primary product, as it is found very persistently in certain products of peptone character which resist hydrolysis by trypsin.*

Aspartic and Glutamic Acids, and Phenyl-alanine.—These products are prepared from the esters of the higher fractions. Phenyl-alanine in the form of the ester can be readily separated from the two other bodies, for whereas the esters of glutamic and aspartic acids are easily soluble in water, phenyl-alanine-ethyl ester is insoluble. Consequently the higher fractions are dissolved in ether, and the ethereal extract is then washed two or three times with water. From this aqueous solution glutamic and aspartic acids are separated after hydrolysis with baryta. The greater part of the aspartic acid can be separated as the barium salt, which is much less soluble than the barium glutamate. Glutamic acid hydrochloride can be readily prepared in

* Cf. reference (8), quoted at the end of this lecture.

a pure form from the mother liquor after separation of the aspartate of barium.

Tyrosine and Cystine.—These bodies, when present in appreciable quantities, generally separate out directly from the liquid, after hydrolysis with hydrochloric acid, and neutralisation of the hydrolysis liquid with caustic soda. They are separated and estimated by Mörner's method already described.*

GROUP H.

Hydroxy-amino Acids.

The two acids of this group which have been identified with the greatest certainty are serine and hydroxy-pyrrolidine-carboxylic acid. Reference has already been made to the constitution of serine (Lecture II., p. 32).

Serine.—This body has recently been discovered amongst the hydrolysis products of several albumens. Abderhalden has isolated it, *e.g.*, from the acids obtained by the hydrolysis of crystallised oxyhæmoglobin of horses' blood. Its ester was obtained in the fraction boiling at 100° under 0.2 mm. pressure, mixed with the esters of phenylalanine, glutamic, and aspartic acids. After separation of the phenylalanine by ether, the hydrolysis of the glutamic and aspartic acid portion by baryta, and separation of the barium aspartate and excess of baryta, it was obtained in the form of the β naphthalene sulpho-derivative by treating the concentrated liquid with β naphthalene-sulphochloride, a reagent first used for isolation of amino acids by Fischer and Bergell.

Hydroxy-pyrrolidine-carboxylic Acid.—This body was first isolated by E. Fischer (5) from gelatine. After the separation of the ether soluble esters of the hydrolysis products in the usual way by Fischer's method (and the repetition suggested by Abderhalden),† the residue containing diamino and oxyamino acids is freed as nearly as possible from salts by repeated extraction with alcohol containing hydrochloric acid, evaporation, re-extraction with alcohol, etc. The nearly salt-free residue is

* See page 29.

† See p. 45.

dissolved in water, and fractionally precipitated with phosphotungstic acid, in order to separate the diamino-acids. Excess of this reagent is used, which is then removed from the solution by excess of baryta, which latter is in its turn precipitated quantitatively by sulphuric acid. From the filtrate, after precipitation of diamino-acids, and the excess of phosphotungstate and baryta, the hydroxyl-pyrrolidine-carboxylic acid (or oxyproline as it has been called), separates out.

Other Hydroxy-amino Acids.—Several new products of this class have been recently isolated by Skraup (21). He introduced a modification into Fischer's experimental method. It was found that the hydrochloride of the esters of the amino acids are soluble in a mixture of alcohol and ether. To the mixture of these hydrochlorides, prepared by Fischer's method, alcohol was added, and the mixture extracted repeatedly with ether. From the hydrochlorides of the alcoholic-ethereal extract the free esters were prepared by Fischer's method, and then distilled.

From the residue, after extraction of the hydrochlorides of the esters, various new hydroxy-amino acids were prepared from caseine. New products were also obtained from the residues of the ester preparation. These various bodies, most of which were obtained only in very small quantities, were prepared by the fractional precipitation or crystallisation of the phosphotungstates and copper salts. The existence of all these bodies cannot yet be considered as definitely proved.

These products are indicated in the list of degradation products given in the last lecture.

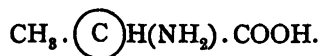
Finally, a body, diamino-trihydroxy-dodecanic acid, has been very recently obtained by E. Fischer (7) from caseine. This body has the formula $C_{12}H_{26}N_2O_6$, and separates with tyrosine, after the hydrolysis of caseine with sulphuric acid, and the quantitative precipitation of the mineral acid with pure baryta. The mother liquors from the recrystallisations of tyrosine were diluted and phosphotungstic acid was added to the acidified solution. After separating the excess of phosphotungstic and sulphuric acids and evaporation, the new acid commenced to

crystallise out. The product which is precipitated by phosphotungstic acid is lysine.

Optical Properties of the Amino-Acids.—It has been already noticed that some acids, *e.g.*, pyrrolidine-carboxylic acids, can be isolated in racemic and optically active forms. It is evident that the acids when originally set free by hydrolysis from the albumen molecule, are optically active, and that racemisation takes place during the continued heating of the acid mixture. This fact increases very considerably the difficulties of isolating and identifying the pure acids, and Fischer, during the course of his researches, often entirely racemised the bodies derived from the esters of any particular fraction by heating them with three times their weight of baryta and twenty times their weight of water for twenty-four hours at 175°C . From the racemised esters he then proceeded to prepare the derivatives necessary for identification, such as those of phenyl-isocyanate. In addition to identification of the acids by this method, E. Fischer has also synthesised various amino-acids in their optically active form (II).

It is not necessary to treat in this place the methods of synthesis of simple amino-acids. Bodies of this class have been known for many years to chemists, and their properties and methods of synthesis have been long familiar, and are discussed in ordinary text-books on chemistry.

Most of these bodies contain an asymmetric carbon atom, as will be evident from the subjoined formula of alanine,



Now the acids, when obtained synthetically, are optically inactive, whereas those obtained by the hydrolysis of albumens are as a rule active. The former constitute the racemic form, a mixture in equal parts of the dextro- and lævo-rotatory forms in equal proportions. [The dextro-rotation of one optically active form is equal to the lævo-rotation of the other.] E. Fischer has devised a method for the preparation of the optically active varieties from the racemic forms obtained by synthesis, with

the object of comparing or identifying them with the optically active bodies obtained from the hydrolysis of albumens. The separation of a racemic variety into optically active constituents has been already accomplished by biological methods, and it is necessary only to refer here to the classical work of Pasteur on the action of moulds on racemic tartaric acid. A similar method has been applied by Schulze and Bosshard (18) to racemic leucine and aspartic acid, and they succeeded in obtaining optically active bodies by means of *penicillium glaucum*.

A more general purely chemical method applicable to acids consists in the preparation of salts of these bodies with optically active alkaloids. These salts can be separated by fractional crystallisation into the optically active antipodes. This method has not succeeded in the case of the amino-acids, which owing to the presence of the strongly basic amino-group do not readily combine with an alkaloid. To obviate this difficulty, E. Fischer has converted the amino-acids into their benzoyl derivatives, and thereby strongly increased their acidic character. On treating these benzoyl derivatives with the strongly optically active bases, brucine or strychnine, salts were prepared. By fractional crystallisation of these salts, the optical antipodes can be separated. On hydrolysing the free optically active benzoyl derivatives with hydrochloric acid, the active acids themselves can be prepared.

For the preparation of the benzoyl derivatives, E. Fischer devised a modification of the Baumann-Schotten method (*i.e.*, treatment of the acid with benzoyl chloride in the presence of caustic alkali), which was found to give unsatisfactory yields. He dissolved the acid in water, and added to the aqueous solution powdered bicarbonate of soda, and afterwards, in small portions at a time benzoyl chloride. The mixture was kept continually stirred at ordinary room temperature for one hour. On acidifying, the benzoyl derivative was precipitated together with benzoic acid (from the excess of benzoyl chloride used). The latter was separated by means of ligroin from the benzoyl acid. As examples of the preparation of an active amino-acid, one or two examples may be given. If benzoyl alanine (65

grams racemic product) be treated with 157 grams of crystalline brucine in hot water, and the mixture be allowed to cool, the brucine salt of *L*-benzoyl alanine separates first. This can be purified by recrystallisation, and yields by treatment with caustic soda, brucine, and the sodium salt of *L*-benzoyl alanine. From the latter the free benzoyl alanine can be prepared by means of acids, and this body on hydrolysis gives the *L*-alanine in a pure state. The mother liquors of the brucine salt of *L*-benzoylalanine contain the dextro-rotatory variety, which can be readily purified by means of the strychnine salt.

By the employment of the principles enunciated above, but varying the details in individual cases, E. Fischer has succeeded in obtaining optically active amino-acids from various synthetically prepared racemic products, and identifying the former with the bodies obtained from the hydrolysis products of the albumens.

GROUP G.

The Hexone Bases.

Diamino-acids, which contain two amino-groups and one carboxyl group have a much more basic character than mono-amino-acids. They can be precipitated by alkaline reagents, such as phosphotungstic acid, and thus readily separated from the mono-amino-bodies.

The first discovery of a basic degradation product from albumens was made by Drechsel in 1889. This investigator described two basic bodies obtained by the hydrolysis of caseine with hydrochloric acid, to which he gave the names *lysine*, $C_6H_{14}N_2O_2$, and *lysatine*, $C_6H_{18}N_8O_2$.

Later researches demonstrated, however, that this latter body described by Drechsel was not a homogeneous product, but a mixture of two bases, viz. *arginine*, a body which had been previously obtained by Schulze and Steiger (19a) from the cotyledons of young germinating plants of *Lupinus luteus*, together with lysine. This discovery is due in the first place to Hedin (12), a pupil of Kossel, to whose systematic work in con-

junction with his pupils (amongst whom one must specially mention Hedin and Kutscher), our knowledge of the basic degradation products is principally due.

Kossel's attention was drawn to this subject in the course of his investigations of the protamines, a class of very basic albumens found combined with a prosthetic group (nucleic acid) in the testicles of various fishes. These bodies we shall have to consider in greater detail in the next lecture. They are important, in that the diamino-acids, which have been found amongst the degradation products of all albumens, are present in their degradation products in very large quantities; in some to the almost entire exclusion apparently of mono-amino-derivatives. The protamines have formed therefore the chief material for the elaboration of the methods for isolating and quantitatively estimating these bases. The methods thus discovered by Kossel and his pupils have been very generally applied to the investigation of the basic products of albumen degradation, in which considerable interest was aroused by the original discovery of Drechsel, which stimulated a large number of other workers to research on this subject, both in his own and other laboratories.

In addition to the bases arginine and lysine just mentioned, must be added a third body, viz., *histidine*, which was described by Kossel in 1893, and prepared by him from the hydrolysis products of the protamines.

These three bodies, viz.,

	Arginine	-	$C_6H_{14}O_4N_2$
	Lysine	-	$C_6H_{14}O_2N_2$
and	Histidine	-	$C_6H_9O_3N_2$

have been called by Kossel the hexone bases, and they occur in varying quantities (one or more) amongst the degradation products of all albumens. Their quantities, relatively to the other degradation products such as the mono-amino-acids, determine to a great extent the chemical character of the albumen, those products, such as the protamines, in which the degradation products contain very large quantities (in some cases over 80 per cent.) of hexone bases being of a distinctly basic character.

It will not be necessary to follow out in detail the various researches of Kossel and his pupils, by means of which our methods for isolation and quantitative estimation have been elaborated. These have extended over several years. Two methods only will be quoted (14 and 15), the one for the isolation and identification of the different products, the other for the accurate quantitative determination.

Older method of Kossel.—In the older method Kossel uses phosphotungstic acid for precipitation of the bases, the reagent employed by Drechsel, by means of which he made his original discovery. The method is a convenient one for the isolation and identification of the bases, if not available for their quantitative estimation.

After the hydrolysis of an albumen, the bases are precipitated by phosphotungstic acid (in acid solution), the phosphotungstate precipitate is filtered off, suspended in water, and baryta added in small quantities at a time to the warmed liquid, with continual shaking until it is present in slight excess. Insoluble barium phosphotungstate is thereby formed, and the free bases pass into solution and are filtered off from the precipitate. The excess of baryta is then precipitated by means of carbonic acid, and after filtering off the barium carbonate, the solution is diluted and saturated with carbonic acid gas. Mercuric chloride solution is then added until the mixture shows an acid reaction. A precipitate is formed, when histidine is present (the mercury hydrochloric acid salt). This is treated with sulphuretted hydrogen in aqueous suspension; the filtrate from the mercuric sulphide thus formed yields histidine hydrochloride on evaporation; this body can be readily purified by recrystallisation.

The filtrate from the mercuric chloride precipitate should contain the two other hexone bases. These, if present, are isolated in the following way. The excess of mercury is precipitated by sulphuretted hydrogen gas, and the excess of hydrochloric acid in the filtrate from the mercuric sulphide, by silver sulphate. After separation of the silver chloride, silver sulphate is added to the hot solution, until a small test portion of the liquid gives a brown instead of a white precipitate with

barium hydrate.* When this point has been reached, baryta is added to main bulk of the liquid till further additions no longer produce a precipitate. The precipitate is then filtered off, washed, suspended in water, and decomposed by sulphuretted hydrogen gas. The liquid containing the free arginine, after filtration from silver sulphide, is neutralised with nitric acid, and evaporated, and yields on standing arginine in the form of its nitrate. The filtrate from the silver arginine contains the lysine. This is freed from the excess of baryta and silver and evaporated to a small bulk. On treatment with an alcoholic solution of picric acid, lysine picrate is precipitated, which on treatment with hydrochloric acid and ether is decomposed, the picric acid passing into the ethereal solution. On evaporation of the aqueous liquid, lysine is obtained in the form of its hydrochloride.

This procedure has been modified in various ways, but the principles, viz., the precipitation of the histidine as mercury salt, the arginine as silver salt, and the lysine as picrate, have been generally retained in the various modifications for qualitative investigation. Kossel and Kutscher (15) have modified the above process, however, in various particulars, and have now elaborated a method by means of which the three hexone bases can be quantitatively estimated in the hydrolysis products of small quantities of albumens. The method is somewhat complex, and reference must be made to the original paper for the details.

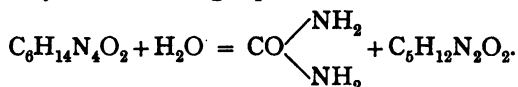
Constitution of the Hexone Bases.

Arginine.—Schulze and Liekernik (19) found that arginine on heating with baryta water yielded urea, and a base which was

* The arginine contains an amino- and also a carboxyl-group. Consequently it can form salts both with acids and metals. On addition of silver sulphate, therefore, we should obtain a silver hydric-sulphate salt, the silver replacing the hydrogen of the carboxyl-group, and the sulphuric acid set free neutralising the amino-group. On adding baryta to the solution of the silver hydric-sulphate, barium sulphate would be formed, together with the silver salt of arginine. This is a white precipitate. If now silver sulphate be added in excess, silver hydroxide would be precipitated, which, owing to its dark colour, would indicate excess of the precipitant.

identical with a product that had been already discovered by Jaffé (13), and called by him ornithine. This body was isolated from the products of hydrolysis of arginine in the form of a benzoyl derivative by the Baumann-Schotten method. This body is known as ornithuric acid, and was originally obtained by Jaffé from the excreta of birds which had been fed with benzoic acid.

The decomposition of arginine into urea and ornithine may be represented by the following equation—



The experiment of Jaffé, who obtained the ornithuric acid by feeding birds with benzoic acid, seemed to indicate that ornithine was a normal decomposition product of the albumens.

Jaffé assumed that ornithine was a diamino-valerianic acid, and this surmise was proved correct by the researches of Ellinger (2), who showed that by means of putrefactive bacteria, ornithine is converted into putrescine, a base of which, owing to its synthesis by Ladenburg (16), the constitution was known.

This can be represented by the formula



The following would therefore be the most probable formula for ornithine—

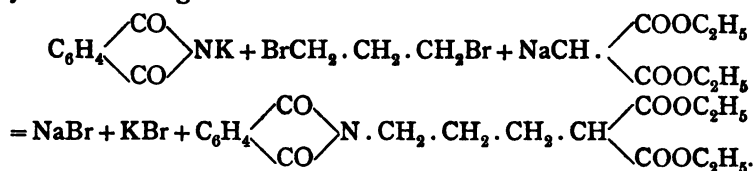
$\text{CH}_2(\text{NH}_2) \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$, α - δ -diamino-valerianic acid, a body which by evolution of carbonic acid gas would readily give putrescine.

The inactive form of α - δ -diamino-valerianic acid has been synthesised by E. Fischer (9) by the following method.

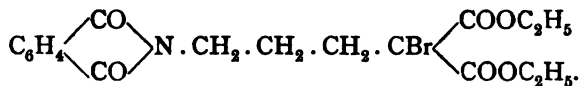
* Potassium phthalimide, propylene bromide, and ethyl

* This body, $\text{C}_6\text{H}_4 \begin{array}{c} \text{CO} \\ \diagup \quad \diagdown \\ \text{CO} \end{array} \text{NK}$, has been largely used for the introduction of amino-groups into the molecule (Gabriel). It readily condenses with halogen derivatives to give bodies containing the group $\text{C}_6\text{H}_4 \begin{array}{c} \text{CO} \\ \diagup \quad \diagdown \\ \text{CO} \end{array} \text{NH}-\text{C}-$, which on hydrolysis at high temperatures split into an amino-body and phthalic acid.

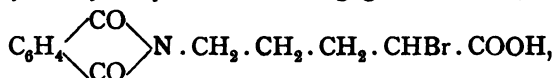
sodio-malonate can be made to condense in a way represented by the following formulæ—



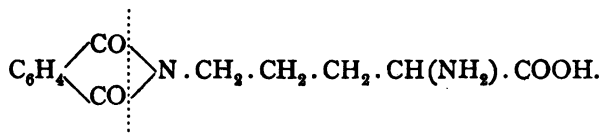
This body on treatment with bromine gives a substance of the formula—



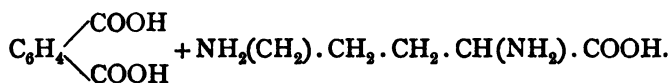
This body on hydrolysis and heating gives an acid,



which on treatment with ammonia gives a diamino-body,

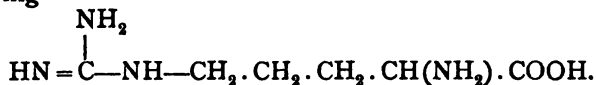


This on hydrolysis at high temperatures yields phthalic acid and inactive ornithine—



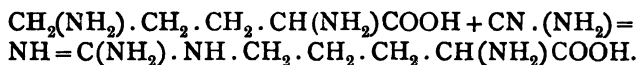
These researches suggest the origin of putrescine formed during putrefaction.

Now arginine yields on hydrolysis urea and ornithine. Hence Schulze and Winterstein (29) assumed that it was a guanidine derivative analogous to creatine. Knowing now the constitution of ornithine, the formula of arginine, on the assumption that it is a guanidine derivative, should be the following—



(ii) The direct synthesis of arginine accomplished by Schulze and Winterstein, who treated ornithine with cyanamide.

The reaction can be represented by the following equation :—

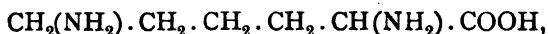


The constitution of arginine may be regarded, therefore, as definitely established.

Lysine.—The constitution of lysine was established by Ellinger (*loc. cit.*) by the same method that he employed in the case of ornithine. He showed that Drechsel's original surmise that lysine is a diamino-caproic acid is correct, as by means of putrefactive bacteria lysine is converted into a base cadaverine, which according to Ladenburg has the following constitution—

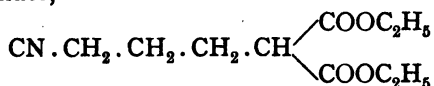


The formula of lysine was therefore assumed to be—

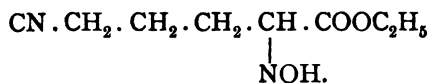


i.e., α - ϵ -diamino-caproic acid.

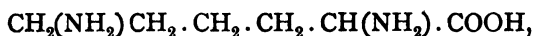
The inactive form of this body was synthesised by E. Fischer and Weigert (10) by the following method. Ethyl cyano-propyl-malonate,



gives on treatment with nitrous acid an oximido-body, viz.,



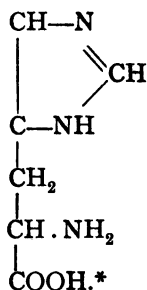
This body on reduction with alcohol and sodium gives



i.e., an inactive lysine.

Hence there is little doubt as to the true formula of lysine.

Histidine.—The constitution of this body is not yet known. The following formula has been suggested by H. Pauly (17):—



We have now considered the methods by which the various nitrogenous degradation products of the simple albumens can be prepared and isolated. We have seen that, thanks to the labours of Kossel and his pupils, the more basic degradation products can be readily prepared and their quantities accurately determined. When we come to consider the mono-amino and other derivatives, the experimental methods are much more complex. The difficulty in dealing with these bodies is due in the first place to the large number of them that have been discovered; it was not in fact till E. Fischer devised the method of fractional distillation of the esters, that a systematic investigation was possible. Even now, all the degradation product of the most accessible albumens have not been isolated. A great deal remains to be done before our knowledge of the carbohydrate groups and the hydroxy-amino-acids is complete. As an example of the application of the various methods described above, to the systematic examination of the degradation products of an albumen, we may quote Abderhalden's researches on the hydrolysis products of oxyhæmoglobin. He obtained the following percentages of hydrolytic products (calculated

* See Appendix.

on the globin, *i.e.*, hæmoglobin without the hæmatin, see p. 122).

	Per cent.	
Alanine	4.19	From one hydrolysis by Fischer's method.
Leucine	29.04	
α -Pyrrolidine-carboxylic acid	2.34	
Phenylalanine	4.24	
Glutaminic acid	1.73	
Aspartic acid	4.43	Determined in separate portion with the tyrosine.
Cystine	0.31	
Serine	0.56	From another portion by Kossel's method.
Hydroxy-pyrrolidine-carboxylic acid	1.04	
Tyrosine	1.33	
Lysine	4.28	
Histidine	10.96	
Arginine	5.42	Determined by Hopkins and Coles' method.
Tryptophane	present	
Total	<u>69.87</u>	

It must be remembered, however, that the amino-acids cannot be obtained quantitatively by E. Fischer's method. From this, one of the most complete investigations of the hydrolysis products on an ordinary albumen yet made, it will be seen that 30 per cent. of the constituent groups remain yet to be determined. Part of these will doubtless be found amongst the carbohydrates and hydroxy-amino-bodies, of which only two, viz., hydroxy-pyrrolidine-carboxylic acid and serine appear in the above list. It may be remarked, however, that Skraup's researches were published after the work of Abderhalden, and no doubt, when the methods for isolation of these bodies have been elaborated, the remaining constituent groups will be determined. Although we are gradually gaining knowledge of the constituent groups, the problem of the constitution of the albumens is by no means nearly solved. To know the constitution, we

must also know the method by which the various groups are conjugated. This problem will be discussed in Lecture VIII., when we consider the chemistry of the more complex degradation products, which are produced by gentler hydrolytic methods than by the use of strong acids.

In the meantime we shall continue the discussion on the simplest degradation products of the albumens, and turn our attention next to the chemistry of the albumens containing prosthetic groups. It is possible that by a further elaboration of the chemical methods, and by the determination of the varying constituents of the different albumens, we may ultimately arrive at a more accurate classification, based on chemical differences, of this class of bodies, and that we shall ultimately be able to discard the more empirical classification now adopted, which is based to a large extent on differences of physical properties, such as precipitability by salts. For this reason we have not attempted to consider the classification of albumens in this course.

REFERENCES—LECTURE III

- (1) BÉNECH and KUTSCHER.—*H.Z.*, **32**, p. 278, 1901.
- (2) ELLINGER.—*Ber.*, **31**, p. 3183, 1899; **32**, p. 3542, 1900; and **35**, p. 2164, 1902.
- (3) E. FISCHER (with HARRIES).—"Apparatus for Distillation in Vacuum."
- (4) E. FISCHER (with ADERS and LEVENE).—"Hydrolysis of Caseine," *H.Z.*, **35**, p. 70, 1902.
- (5) E. FISCHER.—"Preparation of Hydroxy-proline," *Ber.*, **35**, p. 2660, 1902.
- (6) E. FISCHER (with ABDERHALDEN).—"Preparation of Serine," *H.Z.*, **42**, p. 540, 1904.
- (7) E. FISCHER (with ABDERHALDEN).—"Preparation of Hydroxy-amino-dodecanic Acid," *H.Z.*, **32**, p. 540, 1904.
- (8) E. FISCHER.—"Products resistant to Tryptic Digestion," *H.Z.*, **39**, p. 81, 1904.
- (9) E. FISCHER.—"Synthesis of α - β -Diamino-valerianic Acid," *Ber.*, **34**, p. 454, 1901.
- (10) E. FISCHER (with WEIGERT).—"Synthesis of α - β -Diamino-caproic Acid," Prussian Academy, 1902.

- (11) E. FISCHER.—Synthesis of optically active Amino-acids, *Ber.*, **32**, p. 2451, 1899.
- (12) HEDIN.—*H.Z.*, **21**, p. 297, 1895.
- (13) JAFFÉ.—*Ber.*, **11**, p. 401, 1878.
- (14) KOSSEL.—*H.Z.*, **22**, p. 177, 1896 ; and, **26**, 586, 1898 ; and other papers.
- (15) KOSSEL and KUTSCHER.—*H.Z.*, **31**, p. 165, 1900.
- (16) LADENBURG.—*Ber.*, **19**, p. 780, 1886.
- (17) H. PAULY.—*H.Z.*, **42**, p. 515, 1904.
- (18) SCHULZE and BOSSHARD.—*H.Z.*, **10**, p. 138, 1886.
- (19) SCHULZE and LIEKERNIK.—*Ber.*, **24**, p. 2701, 1891.
- (19a) SCHULZE and STEIGER.—*H.Z.*, **11**, p. 43, 1887.
- (20) SCHULZE and WINTERSTEIN.—*H.Z.*, **26**, p. 1, 1898 ; and *H.Z.*, **34**, p. 128, 1901.
- (21) SKRAUP.—*H.Z.*, **42**, p. 274, 1904.

For Hexone Bases, see also articles by Schulze and Winterstein, *Ascher and Spiro's Ergebnisse der Physiologie*, vol. i., 1902.

LECTURE IV

THE PROTAMINES AND HISTONES

NUCLEIC ACID

HAVING considered the methods by which ordinary albumens are degraded into simple products, it is necessary, in order to gain a general knowledge of albumens as a class, that we should study the hydrolysis products of those bodies containing a so-called prosthetic group, which are generally known as proteids.

The most important group of the proteids, perhaps, is that comprising the so-called nucleo-proteids, in which the prosthetic group is a complex group of strongly acidic character, which is known as nucleic acid.

Our first knowledge of the nucleo-proteids as a class is due to the researches of Miescher (9), who turned his attention to the chemical study of spermatozoa. The biological interest attached to this study was probably the chief stimulus to the research, which lead Miescher to separate the heads of the spermatozoa of salmon (which are to be morphologically regarded as the nuclei), and to show that these, after fat-extraction, have the properties of a homogeneous body. This body was shown to be the salt of an organic acid and an organic base, the former, as we shall see almost immediately, a so-called nucleic acid, the latter a protamine.

As we have seen, Miescher in his researches attempted in the first place a morphological separation of the different parts of the spermatozoa, and succeeded in separating the tails from the

heads. For this purpose he employed two different methods, viz.—

(i) He pressed the ripe testicles through muslin, and added to the milky liquid obtained by suspension of the disintegrated substance in water, very weak acetic acid of $\frac{1}{2}$ to 1 per cent. or calcium or barium chloride solutions, and allowed the sediment to settle. This was found to consist chiefly of the heads of the spermatozoa.

(ii) By suspending the disintegrated testicles in water, and centrifuging the mass, with constant renewal of water, re-suspension, and centrifugalisation, Miescher found that the tails were dissolved, and that the spermatozoa heads remained undissolved.

These, on extraction with, first, alcohol, and then alcohol and ether, to dissolve the fats, formed the material on which the first classical researches on the nucleo-proteids were undertaken.

Although Miescher was the first to definitely recognise the true nature of the basic part of the molecule, it is to the researches of Kossel and his pupils that we owe the main part of our knowledge of the protamines. They recognised the albumen-like character of these bodies, and they showed that peptone- and albumose-like products were produced by tryptic digestion, and lastly, by the methods which were discussed in the last lecture, they isolated and identified the degradation products. As we have already seen, these products consist, to a very large extent, of hexone bases, and by reason of their basic character form a well-marked class of compounds.

These protamines have hitherto been obtained with certainty only from the spermatozoa heads of fish.

The principal examples are the following :—

Scombrine, from mackerel.

Salmine, from salmon.

Clupeine, from herring.

Sturine, from sturgeon.

Preparation of a Protamine.

The protamines are now generally prepared by Kossel's method in the form of their sulphates, a procedure which is simpler than that originally proposed by Miescher, who prepared the chloroplatinates and phosphotungstates of the bases, and decomposed these salts in the usual way.

Kossel treats 100 grams of the ripe spermatozoa six times, each time for fifteen minutes, with 500 c.c. of 1 per cent. sulphuric acid. To the acid extract alcohol is added, and the precipitate thus formed is dissolved in hot water; the sulphate of the protamine partially separates as an oil on cooling, the remainder being obtained on concentration of the solution. The salt is purified by conversion into the picrate, which latter compound is reconverted into the sulphate. From this salt the free base can be prepared by means of baryta.

General Characters of the Protamines.

The protamines are all bodies of strongly alkaline reaction. The nitrates and hydrochlorides are easily soluble in water; the sulphates are less soluble. They all give the biuret reaction without the addition of alkali; in fact, freely precipitated cupric hydroxide dissolves in protamine solutions with a violet colour, and on evaporating such solutions to a small bulk and adding alcohol, a violet precipitate is produced. Most of the protamines give no reaction with the reagents of Millon and Adamkiewicz.

They can all be precipitated by the ordinary alkaloidal reagents, and it is *not necessary to have free mineral acids present in the solution*. The protamines, that is to say, are true bases, and not pseudo-bases (see p. 5).

Most metallic salts give precipitates with the protamines; copper sulphate by itself does not. On the other hand, copper sulphate and hydroxylamine in alkaline solution and copper sulphate and sodium hyposulphite produce precipitates of copper salts—reactions which are common to all albumens. Sodium sulphate and sodium chloride precipitate the sulphates from solution. The protamines are not coagulated by heat, and do

not appear to be altered by acids. Salmine is readily diffusible, as is also clupeine hydrochloride; the sulphate of clupeine, however, appears to be indiffusible.

They give characteristic precipitates with nucleic acid; this reaction, in fact, has been suggested as a means of preparing pure protamines, or nucleic acids. The precipitates thus produced are insoluble in water, ammonia, and acetic acid.

Protamines also give precipitates with albumen and albumose solutions which are similar in properties to the histones, a class of bodies we shall have to consider presently.

The empirical formulæ of the protamines is uncertain; as the degradation products contain a relatively large quantity of diamino-acids, the ratio nitrogen : carbon is large.

Degradation Products of Protamines.

Pepsine has practically no action on the protamines. Trypsine, on the other hand, breaks down the protamines into a class of bodies known as the *protones*. These bodies cannot be precipitated from their solutions by salts, and are precipitated by alkaloidal reagents only in acid solutions. They are analogous, probably, to the peptones or albumoses, intermediary degradation products of the albumens, which we shall have to consider later on.

End-products of the Hydrolysis.

Owing to the exhaustive researches of Kossel and his school on the hexone bases, we have accurate methods for the estimation of these bodies, even when only small quantities of material are available for investigation. We are well acquainted, therefore, with most of the degradation products of the protamines produced by hydrolysis with acids; and the main fact which characterises these bodies as a class is the relatively large amount of diamino-acids which can be obtained from them. The main product is arginine. In the cases of salmine, clupeine, and sturine, 82.9-87.8 per cent. of the total nitrogen of the degradation products is in the form of diamino-acids. In the case of cycloptertine the proportion is 67.7 per cent. The

following are some of the results of the analyses of Kossel and Kutscher.

	Percentage derived by hydrolysis.		
	Histidine.	Arginine.	Lysine.
Salmine	o	87.8	o
Clupeine	o	83.5	o
Cyclopteryne	o	67.7	o
Sturine	11.8	63.5	8.4

From the products of hydrolysis attempts have been made to construct formulæ for the various protamine bases. We must remark, however, that whilst the hexone bases form a very large percentage of the degradation products, there are still groups in the molecule which have not been isolated after hydrolysis. The general properties of the protamines may be summarised as follows :—

- (i) They are bodies of albumen-like character.
- (ii) They are very basic, and can be precipitated by alkaloidal reagents even in alkaline solution.
- (iii) They contain a relatively large amount of nitrogen.
- (iv) They yield on hydrolysis small amounts of mono-amino acids, and very large amounts of diamino-acids.
- (v) They yield no cystine or tyrosine groups, except perhaps cyclopteryne. Carbohydrates have not been isolated from their products of hydrolysis.

Kossel has expressed the opinion that protamines are the simplest albumens, and that they form a sort of nucleus existing in all bodies of this class, to which other groups are attached, and that in this way the more complex albumens are built up. Hofmeister, on the other hand, thinks they are the products of change taking place in the albumens during spermatogenesis.

The Histones.

Miescher, in investigating the *ripe* testicles of carp, isolated a product of basic character, to which he ascribed a peptone-like

character. It differed from the protamines in that it gave a very distinct colour with Millon's reagent. Later on, Mathews (8) prepared similar bodies from the spermatozoa of sea-urchins (*arbacine*), and Kossel and Kutscher (5) from the testicles of cod (*gadus-histone*). They have been isolated from bodies other than fish spermatozoa, *e.g.*, from goose-blood, from pus-cells, and from thymus. Various methods for preparing these so-called histones have been employed.

Kossel and Kutscher's Method.—(5) This method was applied to the investigation of the *gadus-histone*. The fat-free spermatozoa were extracted with hydrochloric acid. From this solution the histone was precipitated by saturation with sodium chloride, the precipitate on dialysis redissolved, and on addition of ammonia reprecipitated.

Mathews (8) in preparing *arbacine* extracted the fat-free spermatozoa with 1-2 per cent. sulphuric acid, and precipitated the extract with alcohol. This precipitate was dissolved in water, and the solution then saturated with ammonia; on adding alcohol, the histone was thrown out.

Ehrström (3) used a somewhat different method. The fat-free spermatozoa heads were triturated with concentrated hydrochloric acid, and then three to four times the volume of water was added. By this means nucleic acid was separated. The filtrate was then neutralised with caustic soda. On adding five times the volume of water to the neutral solution, the histone was precipitated; this was purified by repeatedly dissolving in $\frac{1}{2}$ per cent. hydrochloric acid, and precipitating by ammonia.

General Character of the Histones.

The general characters may be summed up as follows :—

(i) The solubility of the different histones in ammonia varies, and is considerably influenced by the salts that are present.

(ii) They give the biuret, the Millon, and the Adamkiewicz reactions; also the reaction of Molisch.

(iii) *They are precipitated in neutral, but not in alkaline*

solutions, by alkaloidal reagents. In alkalis the precipitates are soluble. The histones, then, are more basic than ordinary albumens, but less basic than the protamines, which can be precipitated by alkaloidal reagents in alkaline solutions, whereas ordinary albumens can only be fully precipitated in acid solution.

(iv) They can be salted out from solutions by ammonium sulphate and sodium chloride. (For ammonium sulphate, 4.1 to 4.9 c.c. saturated solution to 10 c.c. of histone solution necessary for precipitation.)

(v) They are not as a rule coagulable.

(vi) They give precipitates with albumens and albumoses, which are soluble in acids, alkalis, and ammonia.

In accordance with their basic character, one might suspect that in nitrogen contents histones would stand midway between an ordinary albumen and a protamine. This in fact is the case.

Arbacine sulphate	contains	15.91	per cent. nitrogen
Lota-histone	"	16.46	" "
Goose-blood histone	"	18.46	" "
(17.48 according to Bang's analysis)			
Thymus histone	"	18.35	" "

Contrast with these the nitrogen contents of a protamine.

Salmine	contains	31.69	per cent. nitrogen
Clupeine	"	25.72	" "

An ordinary albumen contains about 14 per cent.

When we come to examine the degradation products of the histones, we find as we should expect, that they yield more of hexone bases than the ordinary albumen, but less than the typical protamine.

Thymus histone yields 1.79 per cent. histidine, 25.17 per cent. arginine, 8.04 per cent. lysine. An analysis of hydrolysis products of a fish-testicle histone gave 3.3 histidine, 26.9 per cent. arginine, 8.5 per cent. lysine. Compare these numbers with those obtained in the researches on protamines (p. 69) and

on oxyhæmoglobin (p. 62). The intermediary character of the histones is then clearly indicated. We see from these numbers, furthermore, how the hexone-base content influences the general character of the albumen body, and how important it is that we should possess accurate quantitative methods for determining the degradation products of albumens, and thereby establish a chemical basis for their classification.

Other Hydrolysis Products of Protamines and Histones.

Abderhalden and Rona (1) have determined as nearly quantitatively as possible the amino-acids obtainable from thymus histone. They found the following bodies:—

Glycocoll	0.50 per cent.
Alanine	3.46 „
Leucine	11.80 „
α -Pyrrolidine-carboxylic acid	1.46 „
Phenylalanine	2.20 „
Glutamic acid	0.53 „
Tyrosine	5.20 „

Various investigators have examined the degradation products of the protamines with a view to determining the presence of other products. The following have been described (4):—

1. α -Amino-valerianic acid from clupeine.
2. Tyrosine from cycloptertine.
3. Skatol-amino-acetic acid (or the isomer?) from cycloptertine.
4. Pyrrolidine-carboxylic acid from salmine.
5. Serine from clupeine.
6. Alanine from salmine.
7. Leucine from salmine.

These products have not, however, quite the same significance yet as the hexone bases in determining the chemical character. They may, however, prove of considerable importance in separating albumen bodies into smaller groups.

THE PROSTHETIC GROUP OF THE NUCLEINES.

Miescher observed that after the extraction of spermatozoa heads with acids in the preparation of the protamines, a residue was left, which retained the cell form of the spermatozoa heads, had a distinctly acid character, and contained a large quantity of phosphorus (8.2 per cent.). The phosphorus was combined in an organic radical. We have then the basic protamine forming apparently a compound with an organic acid. In examining pus cells by the same method, Miescher also found a residue, which contained somewhat less phosphorus (5.96 per cent.). The same body was also obtained by treating pus cells with pepsine. On examining the chemical properties of this phosphorus-containing body, and comparing it with the one obtained from spermatozoa, Miescher remarked the following differences:—

- (i) The residue from pus cells gave albumen reactions, whereas that from spermatozoa did not.
- (ii) The pus-cell residue, on treatment with strong caustic soda, gave albuminate- or peptone-like bodies;
- (iii) And contained, moreover, about 2 per cent. sulphur.

Hence the residue from pus cells appears to be an albumen-like body. The residue from spermatozoa, on the other hand, did not yield the ordinary albumen reactions.

The difference between the cell-residues—called originally *nucleines* by Miescher—obtained from spermatozoa and pus cells in the course of the preparation of the protamines or histones, was finally explained by Altmann (2), who showed that those albumen-bodies which give albumen reactions, such as the nucleine from pus cells, yield, on treatment with alkalis, or by tryptic digestion, a residue which is quite albumen-free, contains all the phosphorus of the original nucleine, and is in its properties quite similar to the nucleine prepared from spermatozoa. On account of the acid properties, those albumen-free, phosphorus-rich residues were called by Altmann *nucleic acids*. The term *nucleine* can be

retained for the body, which still contain groups giving albumen reactions.*

These nucleines, which can be obtained by action of pepsine on many tissues, are an important class of bodies; it is of interest, therefore, to consider their chemical character in some detail.

Preparation of Nucleic Acid.

The principal group contained in the nucleines is, as already stated, the phosphorus-containing acidic group, viz., the nucleic acid, and various methods have been devised for obtaining bodies of this class in a state of purity, and of constant composition, and quite free from all contaminations giving albumen reactions. The following are some of the chief methods that have been adopted.

Miescher's Method as Modified by Altmann (loc. cit.).—Miescher extracted the fat-free spermatozoa heads with hydrochloric acid until no more protamine went into solution. He then extracted the residue with caustic soda, and precipitated the nucleic acid from this solution with alcoholic hydric chloride. The reactions were carried out at a low temperature to avoid hydrolysis. Tissues from which strongly basic bodies like the protamines are not extracted by hydrochloric acid, Miescher digested first with pepsine, and he then treated the undissolved residues in the same way as he treated those obtained by extraction of spermatozoa heads with hydrochloric acid.

Altmann modified the process in order to free the nucleine entirely from albumen-like groups. The products obtained by Miescher, as has been already noticed, were not always pure nucleic acids, but still contained albumen-like groups. Altmann added to the caustic soda extract acetic acid; this precipitates the nucleins, which have a weaker acidic character; he filtered these off, and then added alcoholic hydrochloric acid. On addition of the strong mineral acid the nucleic acid is precipi-

* There is also another class of phosphorus-rich bodies, which can be obtained by peptic digestion of certain albumens. These differ very greatly in chemical properties from the ordinary nucleins; they are the par-nucleines (see p. 111).

tated. This latter acid is then so strongly acidic in character that it requires a mineral acid to precipitate it from a solution of its sodium salt. The nucleine which has been precipitated with acetic acid is redissolved in caustic soda. It is thereby partly freed from its albumen groups. On adding acetic acid to this solution some nucleine is again precipitated, but on filtering off and adding mineral acid to the filtrate a further quantity of nucleic acid is obtained. By constant repetition of this process the greater part of the nucleic acid can be obtained.

Schmiedeberg's Method (11).—Schmiedeberg has devised a process for the purpose of freeing prosthetic groups from the albumen groups. It has been employed with various modifications not only for the preparation of nucleic acid, but also for obtaining carbohydrate groups from glyco-proteids. It consists in extracting the albumen groups with alkaline copper salts, removing them thus in the form of the violet-coloured body, to which the biuret reaction is due. The nucleic acid is obtained in the first instance as copper salt. As applied to the preparation of nucleic acid from salmon spermatozoa, it was carried out in the following way. The fat-free heads, after extraction with hydrochloric acid, were mixed with copper acetate solution, the solution was filtered, and then strong caustic potash was added. A mucine-like solution is thereby formed, from which nucleic acid in the form of a potash salt contaminated with copper is precipitated by means of alcohol. The greater part of the albumen groups remain in solution in the form of the violet copper compound which yields the biuret reaction. The potassium salt of nucleic acid after filtration from the mother-liquor is purified by re-solution in potash, and reprecipitation by alcohol, and this is repeated until the supernatant liquid no longer shows the violet biuret colour. The potassium salt is then treated with copper acetate again, and the whole of the above processes repeated until a potassium salt quite free from copper and albumen groups is obtained. This is then dissolved in water containing potassium acetate, neutralised with acetic acid, and finally the nucleic acid is precipitated from this solution by hydrochloric acid.

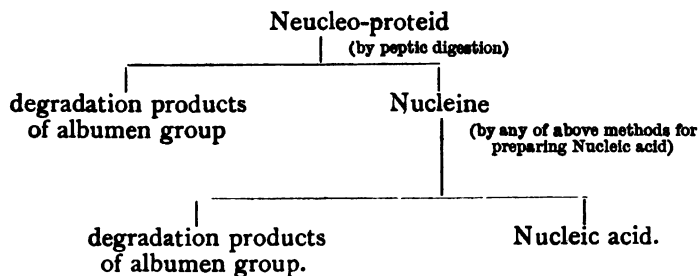
Kossel and Neumann's Method (6).—Kossel and Neumann digested organs containing nucleo-proteids with chloroform water. The gelatinous fluid thus obtained was then strained through a cloth, and treated in small portions at a time with barium hydrate. An impure basic barium nucleate was thereby precipitated. This precipitate was suspended in water, and acetic acid added until the reaction of the mixture was faintly acid. The "neutral" barium nucleate thereby produced was extracted for several days with hot water. The combined aqueous extracts were then thrown into an equal volume of alcohol, whereby nucleic acid was precipitated. This was purified by precipitation from ammoniacal solution by alcoholic hydrochloric acid.

Neumann's Method (10).—The tissue containing nucleo-proteid is dissolved in hot water containing 1.65 per cent. caustic soda and 10 per cent. sodium acetate. In such a solution a tissue rapidly dissolves. The mixture is heated for half an hour on the water-bath, and then filtered through a hot-water funnel. The solution is then evaporated, and neutralised with acetic acid. After filtration from the precipitate which is thereby formed, alcohol is added and the neutral sodium nucleate precipitated. This is purified by dissolving in sodium acetate and reprecipitating by alcohol, processes which are repeated several times. The nucleic acid is finally precipitated from the solution of the sodium salt by hydrochloric acid.

Levene's Method (7).—The principle of this method consists in precipitating the albumen-like bodies with picric acid. It is not employed as much as the method above described.

It is of obvious importance that we should have several methods for preparing bodies like the nucleic acids, for the purity of which we do not possess the ordinary criteria, such as boiling-point, melting-point, etc., as exist in the case of crystalline compounds. It would then be possible to compare the analyses of products obtained by the different methods; and in event of obtaining the same numbers from all the preparations, we should be then fairly certain of having obtained a body which was a chemical entity.

From these results it would appear that we can represent the decomposition of nucleo-proteids by the following scheme :—



In the case of the nucleo-proteids of spermatozoa, the nucleo-proteid appears to be decomposed very readily directly into a protamine (or histone) and nucleic acid.

Properties of Nucleic Acid.

The nucleic acids are amorphous bodies which are but slightly soluble in water. They are soluble in alkalis, especially in presence of potassium acetate (*vide supra* Kossel's method). They can be precipitated from a solution of their sodium salts by strong mineral acid, but by acetic acid only when the solution is saturated with salts. They are therefore strongly acidic in nature. They give precipitates with salts, and also with protamines, whereby a partial synthesis of a nucleo-proteid is accomplished. They also give precipitates with albumens and albumoses, which resemble in properties the nucleines. They diffuse with difficulty through parchment paper.

REFERENCES—LECTURE IV

An admirable monograph on the chemistry of spermatozoa, by Burian has been published in Ascher and Spiro's *Ergebnisse der Physiologie (Biochemische Abtheilung)*, vol. iii., p. 48, 1904. The summary of the chemistry of nucleo-proteids contained in Burian's article has proved of great value in the preparation of this and the succeeding lecture.

(1) ABDERHALDEN and RONA.—*H.Z.*, 41, p. 278, 1904.

(2) ALTMANN.—*Arch. f. Anat. u. Physiol. (Physiol. Abthlg.)*, p. 524, 1889.

- (3) EHRSTRÖM.—*H.Z.*, 32, p. 350, 1901.
- (4) KOSSEL and DAKIN.—*H.Z.*, 41, p. 407, 1904.
- (5) KOSSEL and KUTSCHER.—*H.Z.*, 31, p. 188, 1900-1.
- (6) KOSSEL and NEUMANN.—*Ber.*, 27, p. 2115, 1894.
- (7) LEVENE.—Several papers, *H.Z.*, 1902-3.
- (8) MATHEWS.—*H.Z.*, 23, p. 399, 1897.
- (9) MIESCHER.—Collected letters ; also, *A.P.P.*, 37, p. 100, 1896.
- (10) NEUMANN.—*Arch. f. Anat. u. Physiol. (Physiol. Abthlg.)*, p. 374, 1898 ; and supplementary volume, p. 552.
- (11) SCHMIEDEBERG.—*A.P.P.*, 43, p. 57, 1900.

LECTURE V

THE DEGRADATION PRODUCTS OF NUCLEIC ACIDS

By very gentle hydrolysis with acids or alkalis, intermediary decomposition products of nucleic acid have been obtained. Such products are richer in phosphorus than the acid from which they have been prepared. Neumann found, for example, that when a nucleo-proteid is treated with caustic soda and sodium acetate according to his method for preparing nucleic acid, and the mixture is heated for two hours, instead of an hour and a half, a somewhat more soluble product is obtained. This he calls *b*-nucleic acid, and he suggests that it is a depolymerised nucleic acid. Ordinary nucleic acid appears, furthermore, to undergo some decomposition on treating with water at 60°. A product which is fairly readily soluble in water is thereby obtained. Various intermediary degradation products have been described by different authors, such as the nucleo-thymic and thymic acids of Kossel and Neumann. Our knowledge of these bodies is, however, still very incomplete, and we shall proceed immediately therefore to the consideration of the simple degradation products, and the methods by which they can be obtained. For our knowledge on these subjects, we are again indebted to the researches of Kossel and his pupils.

The following groups of degradation products have been obtained :—

- (i) Formic acid.
- (ii) Ammonia.

- (iii) Phosphoric acid.
- (iv) Carbohydrates, or carbohydrate derivatives.
- (v) The purine bases.
- (vi) The pyrimidine bases.

Nucleic acids of different origin yield different degradation products, all of which, however, belong to the above group.

GROUPS I. and II.

The exact relation of the ammonia which is obtained in the form of salts by acid hydrolysis to the molecule is not accurately known. It is possibly only a secondary product obtained by the action of acids on the purine base. The same may be said of formic acid.

GROUP III.

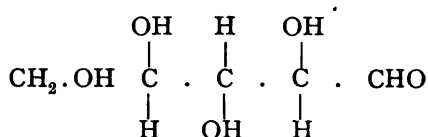
Phosphoric acid is obtained in considerable quantities by the hydrolysis of nucleic acids. It forms an important part of the molecule, and we shall reserve discussion on this subject till we consider the question as to how the various groups are conjugated to form the nucleic acid molecule.

GROUP IV.

The Carbohydrates obtainable from Nucleic Acids.

Kossel (5) first showed that by the hydrolysis of a nucleic acid a carbohydrate-like body, capable of reducing Fehling's solution, and yielding an osazone melting at 156° , could be produced. The nucleic acid used in these researches was that obtained from yeast. Almost at the same time, Hammersten (4) isolated from the hydrolysis products of pancreas nucleoproteid a carbohydrate which gave an osazone melting at 158° - 160° , and which Bang subsequently showed was derived from the nucleic acid. This carbohydrate was subsequently

identified as *L*-xylose, a five carbon-sugar, by Neuberg. Its constitution is represented by the formula—



Neuberg (8)* identified the body by the following method. The expressed pancreas juice was boiled with dilute hydrobromic acid for some hours, and the mixture was then neutralised with lead carbonate and filtered off from the precipitate of lead bromide thus formed. The filtrate was then evaporated to dryness and extracted with alcohol. After evaporating off the alcohol from the extract, an impure syrup was left. This was then subjected to oxidation with bromine water; an acid was thereby formed. This was separated from the hydrobromic acid by a fractional precipitation and crystallisation of the lead salt. From the fraction of lead salt containing the oxidation product the free acid was obtained by means of sulphuretted hydrogen. This was converted into its brucine salt, which can be readily recrystallised. It was found to be identical with *L*-xylic acid, a body which can be directly prepared from *L*-xylose by oxidation with bromine water.

It is not known yet whether all nucleic acids yield pentoses on hydrolysis; it is probable, however, that hexoses are obtainable from some of the nucleic acids, for some of the spermatozoa acids, as well as thymo-nucleic acid, yield, as Kossel and Neumann have shown, when heated with 30 per cent. sulphuric acid, the body lævulinic acid. This latter is a characteristic decomposition product of the hexoses.

The methods for isolating the carbohydrate groups from the degradation products of nucleic acid are by no means entirely satisfactory; as in the case of the degradation products of the albumens, a great deal of experimental work remains to be done before the facts bearing on this group in these complex molecules are definitely settled.

* Compare p. 26.

GROUP V.

The Purine Bases.

The methods for the isolation of this important class of bodies from nucleic acid are due principally to Kossel.* He succeeded in isolating from various nucleo-proteids four of these bodies, viz. :—

Guanine	-	-	$C_5H_5ON_5$
Xanthine	-	-	$C_5H_4O_3N_4$
Hypoxanthine	-	-	$C_5H_4ON_4$
Adenine	-	-	$C_5H_5N_5$

As an example of the method, the following preparation of the purine bases from pancreas may serve. 75 lbs. of pancreas are heated with 200 litres of dilute sulphuric acid, containing 0.5 parts concentrated acid to 100 parts of water, for three to four hours. At the end of this time the sulphuric acid is quantitatively precipitated by baryta, and the barium sulphate is then filtered off. To the filtrate is then added silver nitrate and ammonia, which produces a precipitate of the purine bases. This is then filtered off, and dissolved in hot nitric acid in the presence of urea (the latter body inhibiting the action of the nitrous acid formed). Guanine, hypoxanthine, and adenine then separate from this solution on cooling, in the form of their silver salts. These, after filtration, are decomposed by sulphuretted hydrogen under pressure. The filtrate from the silver sulphide is then concentrated, and then saturated with ammonia gas on the water-bath. Adenine and hypoxanthine go into solution, whilst guanine remains undissolved. This latter body is then filtered off, and the filtrate is evaporated, when the adenine separates out first.

We now come to consider the general chemistry of the purine

* Reference must be made here to the important researches of Salomon and Krueger (10) on the isolation of purine bases from urine. Their work has materially added to our knowledge of the methods for isolating purine bases : investigators working on nucleic acids have been able to avail themselves of these methods.

bases. These bodies are of great interest from the physiological and pathological standpoint, and have been the subjects of a very large number of researches ; there is, in fact, scarcely a topic in physiological chemistry which occupies so large a space in literature. The attention paid to these bodies by physiologists is justified, moreover, by the important part they play in general metabolism, and in the pathology of gout.

The name of "purine" bases is of recent origin, and due to E. Fischer (2), by whose researches the chemical relationships of these bodies to one another and to the most important member of the group, viz., uric acid, have been definitely established.

A short account of the historical development of our knowledge of the chemistry of these important bodies is of interest, as with the researches of E. Fischer on this subject a chapter in organic chemistry may be said to be closed.

History of the Researches on Uric Acid.

Uric acid, the first known member of the group, was discovered by the Swedish chemist, Scheele, in 1776, in urine ; it was also discovered independently about the same time by another Swede, Bergmann, who isolated it from bladder stones. The next investigator of note who made observations on this subject was Pearson (*Philosophical Transactions of the Royal Society*, 1798), who isolated uric acid from gouty concretions. A little later it formed the subject of a series of researches by Fourcroy, who made the important discovery that uric acid on treatment with hydrochloric acid decomposes, yielding amongst its products of degradation urea.

In 1805 Fourcroy and Vauquelin discovered a new source of uric acid, when they showed that the excretion of birds contained 25 per cent. of this body, and to-day it is most readily obtained in large quantity from Peruvian guano.

The elementary composition was first demonstrated by Liebig in 1834, and simultaneously and independently by Mitscherlich. The formula assigned was $C_5H_4N_4O_3$.

Whilst Liebig was investigating the chemistry of uric acid, Wöhler was also occupied with researches on the same subject. He had shown in 1829 that its product of destructive distillation, which had been discovered by Scheele and called by him "pyruvic acid," was identical with cyanuric acid, a body which had been synthesised by Serullas in the previous year; he showed that urea was formed at the same time.

Liebig and Wöhler, shortly after the above discoveries, joined forces, and by their joint labours great progress was made towards our knowledge of the chemistry of uric acid. They together systematically studied the oxidation products; amongst the most important discoveries they made was the fact that uric acid on oxidation with lead oxide gives a body, *allantoine*, which had been previously isolated from the allantoic fluid of cows. They also studied systematically the oxidation products produced by treatment with nitric acid, and succeeded in preparing in the course of their investigations a large number of new bodies in a pure state. Amongst these may be mentioned the following—alloxantine, alloxuric acid, thionuric acid, uramil, oxaluric acid, parabanic acid, mycomelanic acid, and uramilic acid. Liebig and Wöhler also studied the well-known mur-exide reaction, which had been previously discovered by Prout (*Phil. Trans.*, 1828).

After Liebig and Wöhler had ceased their researches, a long interval elapsed before further progress was made. The degradation products had been thoroughly studied, and chemists were awaiting the development of synthetical methods; until such methods had been elaborated, it was almost impossible to gain any definite conception of the structure even of the simpler degradation products.

It was not until the years 1863 and 1864 that any fruitful researches on the synthesis of bodies derived from uric acid were brought to a conclusion. In these years Baeyer and Schlieper published memoirs in which they showed that uramil on treatment with potassium cyanate yields the so-called pseudo-uric acid. Baeyer further showed that allantoine yields on reduction a body called by him *hydantoine*, which he syntheti-

cally prepared from bromacetyl urea. This was the first synthesis of a body of the parabanic acid group.

A few years after, Grimaux accomplished the synthesis of various other bodies of this group, viz. :—

Parabanic acid from oxaluric acid.

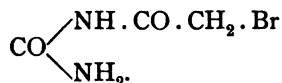
Barbituric acid from malonic acid and urea.

Allantoine from urea and glyoxylic acid.

Pyruvil derivative from pyrotartaric acid and urea.

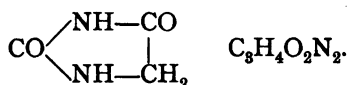
From the researches carried out to this date, some conception could be formed as to the constitution of uric acid; in order to understand the progress, it will be necessary to give the formulæ representing some of the synthetical reactions.

Hydantoine.—The first synthesis of these bodies was that of hydantoine from bromacetyl urea. The formula of this body was known without doubt, viz.,

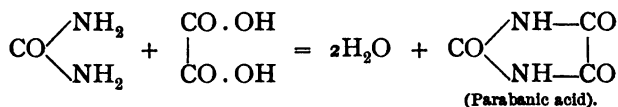


On treatment with alkalis it is converted into hydantoine with scission of hydrobromic acid.

Hydantoine would then have the formula

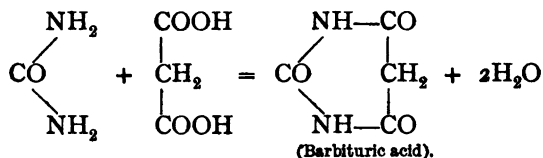


Parabanic Acid.—The next synthesis was that of a direct oxidation product of uric acid, first obtained by Liebig and Wöhler, viz., parabanic acid. This body was synthesised by treating a mixture of urea and oxalic acid with a powerful dehydrating reagent, viz., phosphorus oxychloride. The inter-reaction of these two bodies can be represented as follows :—



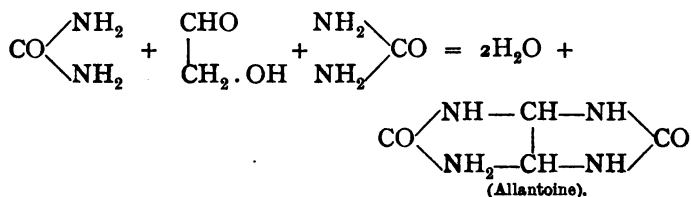
This body is a type of a class of urea condensation products known as the ureides.

Barbituric Acid.—This body was obtained from alloxantine by heating the latter with concentrated sulphuric acid. It also is an ureide, and Grimaux obtained it by heating the next higher homologue of oxalic acid, viz., malonic acid, with urea.



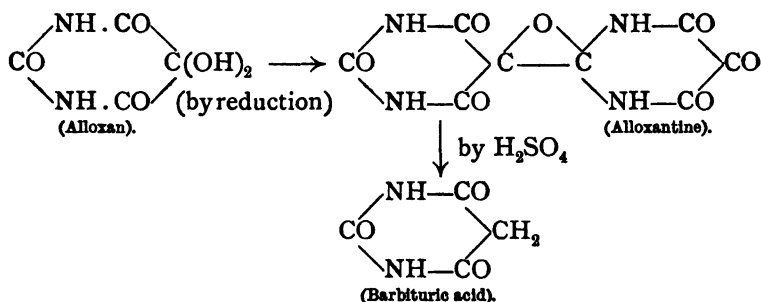
The above are all examples of monureides, *i.e.*, bodies formed by condensation of one molecule of urea with one molecule of acid.

Allantoine.—The synthesis of its reduction product hydan-toine by Baeyer gave some clue to the constitution of this body. It was obtained originally as a direct oxidation product of uric acid by Liebig and Wöhler, and was synthesised by Grimaux by heating glyoxalic acid with urea to 100°. In this case *two* molecules of urea condense with *one* molecule of acid.

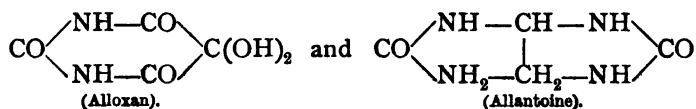


Alloxan and Alloxantine.—Finally, we must consider the formula of another important oxidation product of uric acid, viz., alloxan. This body on gentle reduction in the cold is converted into alloxantine, which on digesting with sulphuric acid is converted into barbituric acid, of which the synthesis is given above.

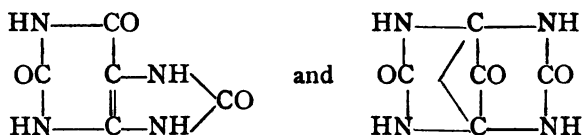
These reactions leave little doubt as to the formulæ.



At this stage of the researches, then, we have sufficient data for forming some conception as to the constitution of uric acid, for we know without doubt the constitution of two important oxidation products, viz.,



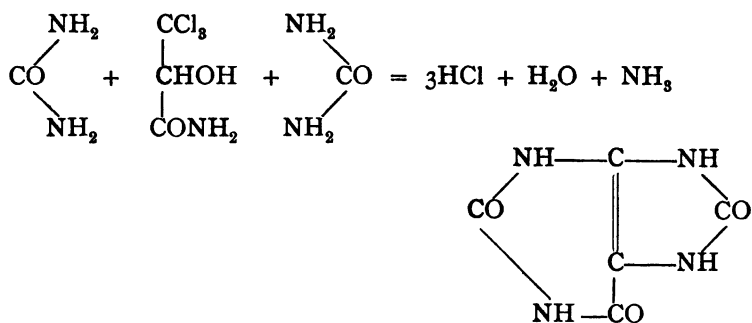
The latter of these bodies contains only one carbon atom less than uric acid. Further, the constant occurrence of ureides amongst the oxidation products of uric acid suggested that this body was a diureide. The possible formulæ now for the bodies capable of yielding two oxidation products of the constitution represented above is very limited, and in fact only two were suggested, viz.,



The former was suggested by Medicus and the latter by Fittig. The time was now ripe for an attempt to synthesise uric acid itself. This feat was first accomplished by Horbaczewski, who, remembering some results obtained by Strecker in 1868 (who showed that glycocholl could be obtained by the degradation of uric acid), synthesised the acid by the direct condensation of

urea and glycocholl (1882). Very poor yields were obtained by this method, and no definite conclusion could be drawn as to the constitution.

A few years later Horbaczewski accomplished another synthesis, viz., by the condensation of the amide of trichlorolactic acid with urea—

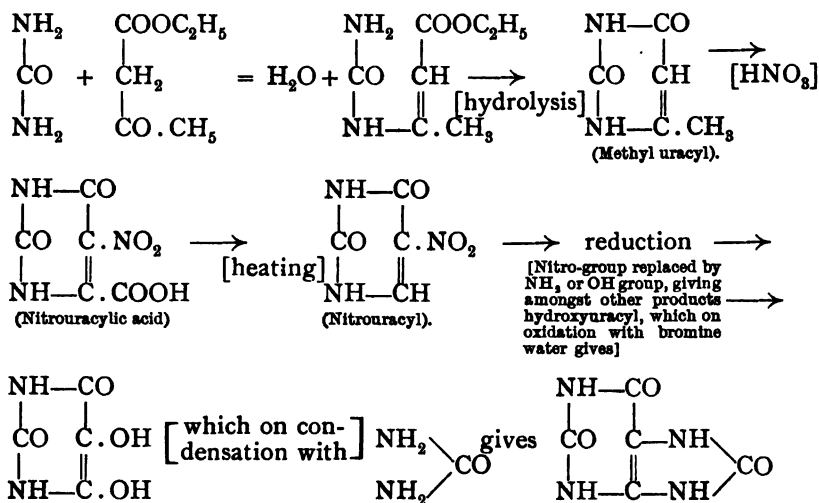


Although this synthesis does not definitely decide between the formulæ of Medicus and Fittig, it is of considerable physiological interest. It is known that lactic acid is a product of muscular activity, and it is suggested that uric acid may be synthesised somehow in the body from urea and lactic acid. If formed there in abnormally large quantities, it may arise in certain cases from synthetical processes in the body. On the other hand, it may be conceivably formed from other purine bases, such as xanthine, hypoxanthine, etc., in the organism, products which we shall see later on can be prepared by the degradation of nucleic acid. There are therefore two conceivable methods by which uric acid can be formed in pathological cases, viz., by abnormal degradation of nucleic acid, a catabolic process, or by abnormal synthesis, an anabolic process. Both these chemical possibilities must be taken into account; then, in studying the pathogenesis of gout.

The constitution of uric acid was finally settled by a synthesis of Behrend and Roosen in 1885, by a method which left no doubt that the formula suggested by Medicus, and not that of Fittig, correctly represented the constitution of uric acid.

Briefly, this synthesis is as follows.

Urea condenses with ethyl acetoacetate to form a product which on hydrolysis yields methyl uracyl. This latter body on oxidation with nitric acid gives nitrouracyl, which on heating readily evolves carbonic acid to give nitrouracyl. Nitrouracyl on reduction in presence of acids yields a mixture of amino- and hydroxy-uracyl, which latter body on oxidation with bromine water forms dihydroxyuracyl. The body condenses directly with urea in the presence of sulphuric acid to form uric acid. Formulæ—



Later on, in 1896, Fischer and Ach obtained uric acid from pseudo-uric acid by heating the latter with hydrochloric or oxalic acids. The synthesis was used as a general method by E. Fischer for the preparation of other bodies from substituted pseudo-uric acids, and will be described presently, when discussing Fischer's researches.

We see now that our knowledge of the constitution of uric acid was only acquired by a long series of researches by many investigators, extending over a century.

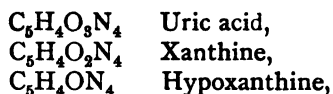
Purine Bases.—In the meantime other bodies were dis-

covered, which on investigation proved to be closely allied to uric acid, not only from the analysis numbers and the empirical formulæ derived therefrom, but from their similarity of origin (*e.g.*, from urine, bladder stones, guano), and from the fact that oxidation products of the uric acid group of bodies (such as alloxan and parabanic acid, etc.) were obtained from all.

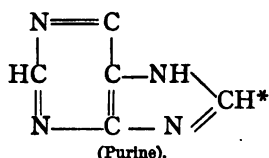
In 1817, Marcet discovered xanthine in bladder stones; in 1845, guanine was discovered by Unger in guano; in 1850, hypoxanthine was discovered by Scherer; in 1885, Salomon isolated monomethylxanthine (heteroxanthine) in the urine, and in the same year Kossel described the important product adenine, obtained from the nucleo-proteid of pancreas.

The relation of all these bodies to one another was finally determined by the researches of E. Fischer and his pupils, and it is to him that we owe the modern generic name of "purine bases," a much more convenient expression than "alloxuric" or "xanthine bases," by which they were formally known.

Fischer's Researches.—If we compare the empirical formulæ of three of these bases, viz.,

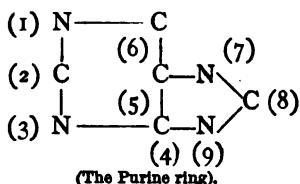


a chemical relationship is immediately suggested. It is possible that they are all oxy-derivatives derived from an oxygen-free body of the formula $C_5H_4N_4$. To such a body E. Fischer gave the name of purine. Assuming that this body was the parent substance of uric acids and allied bodies, and knowing the constitution of uric acid, the following formula was assigned to it—

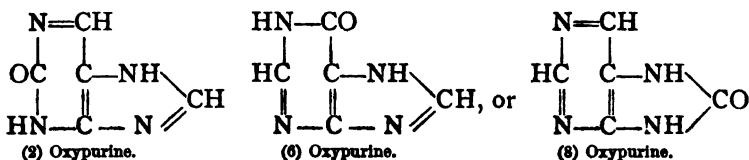


* This is the simplest oxygen-free body containing the purine ring. It will be noted that isomeric forms are theoretically capable of existence. The nature of isomers of this type, however, has not been investigated.

It has not been prepared from natural products, but was synthesised by Fischer in the course of his researches. It contains the following ring, to the members of which Fischer assigned the numbers given below—



If, now, we commence to derive isomers from this type, we shall see that three mono-oxypurines are theoretically possible—

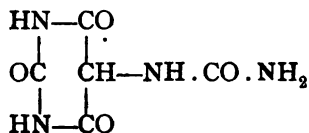


From this example alone of the difficulty of assigning the correct formula to a purine derivative, it is at once perceived we have cases of isomerism considerably more complex than those in the benzene derivatives. It is by the methods elaborated by E. Fischer's researches that the various isomers in the purine series can be prepared, and we can determine, for example, which of the above formulæ represents hypoxanthine, which is presumably an oxypurine, and to which carbon atoms in the purine ring are attached the oxygen atoms in the xanthine molecule.

In order to determine the constitution, and to synthesise the numerous possible derivatives of the purine group, five different types of reaction were employed by Fischer.

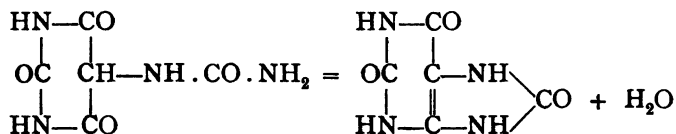
1. The synthesis from pseudo-uric acid.

Pseudo-uric acid has the following formula—



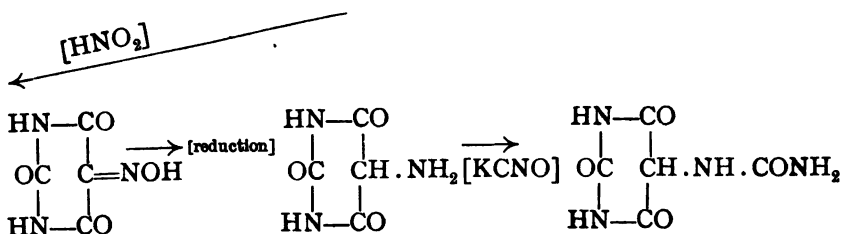
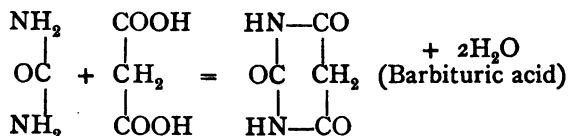
92 DEGRADATION PRODUCTS OF NUCLEIC ACIDS [LECT.

Fischer and Ach have found that pseudo-uric acid on treatment with hydrochloric acid, or oxalic acid at higher temperatures, is readily converted into uric acid.



Pseudo-uric acid can itself be synthesised in the following way :—

On heating urea with malonic acid and phosphorus oxychloride to 100°, a ureide, malonyl urea (barbituric acid) is obtained. This body, as it contains a —CH₂— group between two carboxyl groups, readily reacts with nitrous acid to give an oximido-derivative. This latter body can be reduced to the corresponding amino-derivative, from which, by treatment with potassium cyanate, pseudo-uric acid is obtained. The successive stages are indicated by the following formulæ :—



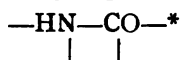
The importance of this synthesis can be recognised at once, for if instead of condensing malonic acid and urea we condense malonic acid and a substituted urea, such as methyl urea, we can synthesise directly a substituted uric acid. Substituted malonic acids can also be employed. In this way various amino- and methyl-derivatives have been prepared.

2. The second type of reaction was the preparation of alkyl-derivatives by means of an alkyl iodide on a purine derivative in the presence of alkalis. This reaction was important for the preparation of various methyl purine derivatives like caffeine, theobromine.

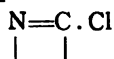
These bodies do not concern us directly here, as they do not occur amongst the degradation products of nucleic acid.

3. From a uric acid derivative various chloro-bodies can be prepared by the action of reagents like phosphorus pentachloride or phosphorus oxychloride.

From a body with the group



a body containing the group

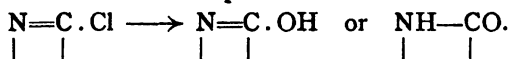


can be prepared.

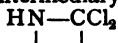
This particular reaction was worked out in great detail by E. Fischer. In a body like uric acid there are three groups in which chlorination can take place. Fischer found that he could obtain different products by varying the conditions of experiment. By using higher temperatures, for example, he found that more of the HN—CO groups were attacked than at low

temperatures. Furthermore, different results were obtained by using different chlorinating reagents, such as the oxychloride, the pentachloride, or mixtures of these bodies. In this way a large number of derivatives were prepared. From uric acid, for example, it is possible to prepare trichlorpurines, dichloroxypurines, and monochlorodioxypurines.

4. By treating these chloro-bodies with aqueous alkalis or acids, the chlorine could be replaced—



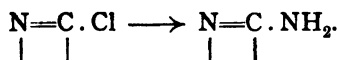
* On the assumption of the intermediary formation of the group



from which a scission of hydrochloric acid readily takes place.

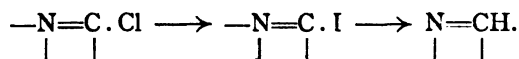
Furthermore, by varying the conditions of hydrolysis, etc., one or more chlorines could be replaced. This reaction is the reverse of reaction 3.

Again, by treating the chloro-bodies with ammonia at 150°, amino-derivatives could be prepared. Thus,



By potassium hydrosulphide, thio-derivatives corresponding to oxypurines could be obtained.

5. The chlorine in the chloro-bodies can be replaced by hydrogen. By treatment with hydriodic acid at 0° an iodo-body can be prepared, which latter product can be readily reduced by zinc dust, the hydrogen replacing the iodine—

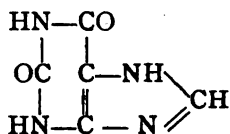


By intermediation, therefore, of the chloro-derivatives, we can pass from a polyoxypurine derivative to one containing less, or even no oxygen.

E. Fischer, in the course of his researches, extending over many years, prepared a very large number of derivatives, of which he gives a list in his general paper on the subject. By passing from one body to another, and by studying the degradation products of the different bodies obtained, he gradually succeeded in orientating the groups, and determining the constitutions, very much in the same way that Kekulé and his followers determined the constitution of the numerous benzene derivatives.

We cannot, of course, consider these reactions in detail, but may take a few examples which apply to the degradation products of nucleic acid and serve to illustrate the methods.

Xanthine.—This body was found to be a dioxypurine of the constitution



On treatment with hydrochloric acid, alloxan and urea were formed. This immediately suggested the above formula.

Xanthine could be synthetically obtained from uric acid by intermediation of trichlorpurine, which is obtained by the most drastic method of chlorination from the former body.

Two methods were employed for preparing xanthine from trichlorpurine.

(a) By the action of sodium ethoxide, which gives diethoxy-chlorpurine. This latter body on treatment with hydriodic acid is converted into xanthine.

(b) By the action of hydriodic acid at 0°. This yields di-iodopurine, which on hydrolysis with hydrochloric acid gives xanthine.

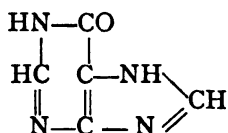
Theobromine is dimethylxanthine (3.7).

Paraxanthine (from urine) is dimethylxanthine (1.7).

Theophylline is dimethylxanthine (1.3).

Caffeine is trimethylxanthine (1.3.7).

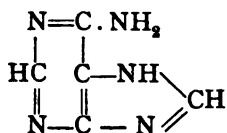
Hypoxanthine.—This body is 6-oxypurine, with the formula



This can be obtained from trichloropurine also. The latter on treatment with alkali under suitable conditions, yields *6-oxy-2.8-dichloropurine, from which, on replacement of chlorine atoms by hydrogen, hypoxanthine is obtained.

Furthermore, hypoxanthine can be prepared from adenine by means of nitrous acid.

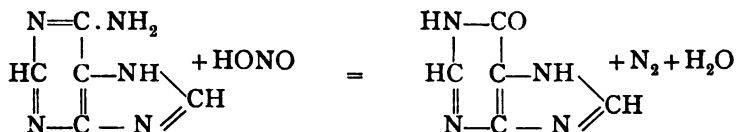
Adenine.—Adenine is 6-aminopurine—



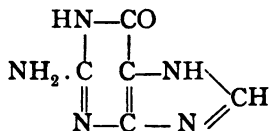
* The formulæ of these bodies were determined in the course of the orientation researches on the chloro- and chloroxypurine-derivatives.

96 DEGRADATION PRODUCTS OF NUCLEIC ACIDS [LECT.

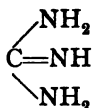
This body has been obtained from uric acid by two methods. The simplest is the following. Trichloropurine, on treatment under certain conditions with ammonia, yields 6-aminodichloropurine, which on reduction yields adenine. The formula of this body is also determined, from the fact that with nitrous acid it is readily converted into hypoxanthine—



Guanine.—This body is 2-amino- 6-oxy-purine—



On treatment with chlorine in hydrochloric acid solution it readily yields guanidine—



There is therefore no doubt as to the position of the amino-group in this body. Furthermore, it is readily converted into xanthine by nitrous acid, a reaction of considerable use in originally determining the position of at least one oxy-group in this body.

In addition to the above bodies, the purine group also contains a series of methyl derivatives, such as caffeine, theobromine, etc., which have been isolated from tea, coffee, etc. The relation of these to other purine derivatives have also been studied by means of the various reactions described above.

GROUP VI.

The Pyrimidine Bases.

Our knowledge of these bodies is also due chiefly to the researches of Kossel and his pupils.

The three principal bodies of the group are—

Uracil.

Thymine.

Cytosine.

They can be prepared from nucleic acid by hydrolysis with acids; it requires, generally, however, a more drastic treatment to obtain them than is necessary for the preparation of the purine derivatives. For their preparation, a nucleic acid is heated for two hours at 150° with 20-30 per cent. sulphuric acid.

Thymine was isolated simultaneously and independently by Kossel and Neumann and by Miescher. The latter obtained it from the nucleic acid of salmon spermatozoa in the filtrate from the phosphotungstic acid precipitate of the decomposition products of the acid; he freed the filtrate from excess of the precipitant by baryta in the usual way, and found that on concentrating, a new base separated, to which he gave the name "nucleosine."

Kossel and Neumann, in working on a nucleic acid prepared from thymus glands, showed that this body could be decomposed by gentle hydrolysis into, amongst other products, adenine, and an acid which they isolated by precipitating it in the form of its barium salt from aqueous solution by means of alcohol. This acid, which they designated thymic acid, appeared to be a body of some complexity. They subjected it to hydrolysis by boiling it with 30 per cent. sulphuric acid. They obtained thereby a considerable quantity of a hitherto unknown base, which readily separated out in a crystalline form after freeing the hydrolysis products from excess of sulphuric acid by baryta. In some later researches they investigated the hydrolytic action of various strengths of sulphuric acid, and found that if they employed 20 per cent. sulphuric acid at a

G

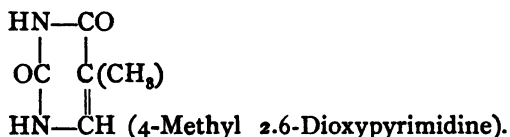
temperature of 150° for two hours, the adenine formed normally was entirely destroyed. They examined the basic products of hydrolysis obtained under these conditions, and found two bodies, viz., one giving an insoluble and the other a soluble phosphotungstate. The latter was found to be thymine. After freeing the filtrate from the insoluble phosphotungstate from excess of the precipitant, mercuric nitrate and caustic soda were added. A mercuric salt was thereby precipitated. The free base obtained from the mercuric salt was reprecipitated by silver nitrate containing free ammonia; from this silver salt thymine was obtained pure. The insoluble phosphotungstate was decomposed by baryta. The filtrate from barium phosphotungstate was warmed, to drive off the ammonia; excess of baryta was quantitatively precipitated by sulphuric acid, and the filtrate from the barium sulphate evaporated. On adding ammonia, a new base called *cytosine* separated.

Kossel also turned his attention to the nucleic acids of vegetable origin, and one of his pupils, Ascoli (1), investigated the degradation products of a vegetable nucleic acid. On proceeding by the method necessary for isolation of thymine, he succeeded in preparing a third base, viz., *uracil*. This was the principal product isolated, the body thymine being obtained only in very small quantities. After the discovery of the three bases, thymine, cytosine, and uracil, investigations were made with the object (a) of determining the relative quantities of these bodies obtainable from the different nucleic acids, in order to quantitatively distinguishing between the acids derived from various sources, such as the nucleic acids of yeast, thymus gland, pancreas, and spermatozoa; (b) of determining the chemical nature of these bases.

Various methods have been devised for the quantitative separation; for this purpose fractional precipitation in acid solutions with mercuric sulphate, precipitation of phosphotungstates, picrates and silver salts, and other means have been applied. We are indebted to Kutscher, Kossel and Steudel (6), and to Levene (7), for various processes; for the details, reference must be made to their papers.

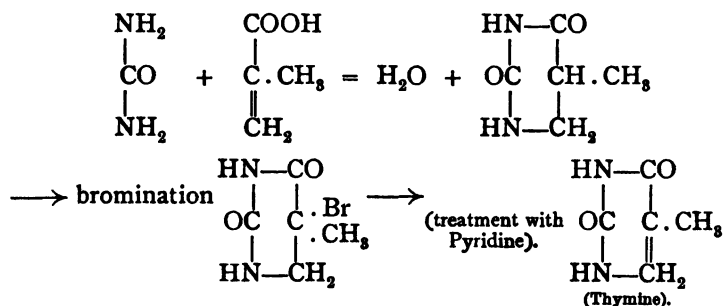
Constitution of the Pyrimidine Bases.

Thymine.—The constitution of this body was determined by Steudel (11), who assigned to it the formula—



He found that it yielded on oxidation urea; he also found that on nitration it yielded a nitro-body which, on reduction with tin and hydrochloric acid, gave the alloxan reaction. The presence then of a pyrimidine ring was therefore probable.*

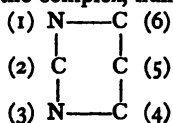
It has been synthesised by E. Fischer and Roeder in the following way. Urea is heated with methyl acrylic acid to 210°-220° C. A ureide is thereby produced. On treating this body with bromine in acetic acid at 100°, a monobromo-derivative is obtained. On boiling this with pyridine, scission of hydrobromic acid readily takes place with the formation of thymine. Formulæ—



Another synthesis has also been carried out by Wheeler and Merriam.

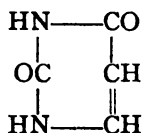
* For formula of alloxan, see p. 87.

Pyrimidine rings contain the complex, numbered as follows :—



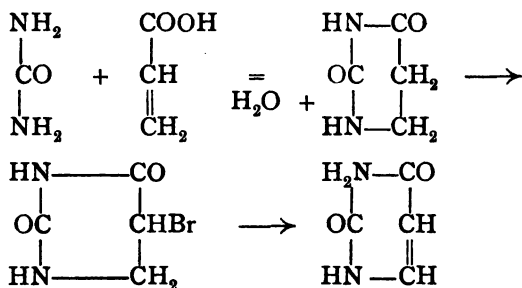
100 DEGRADATION PRODUCTS OF NUCLEIC ACIDS [LECT.

Uracil.—The constitution of this body has also been determined. It has the formula—



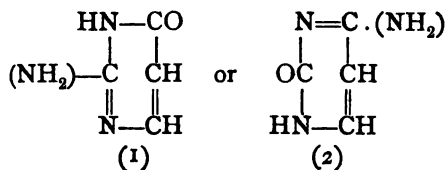
Thymine is therefore methyluracil.

The fact that this body gives certain alloxan reactions suggested the above formula. It was also synthesised by Fischer and Roeder (3), by a method analogous to that used for thymine. Urea was condensed with acrylic acid, and a mono-ureide thereby prepared. This on treatment with bromine yielded a monobrom body, from which, by scission of hydrobromic acid, uracil was obtained. Formulæ—



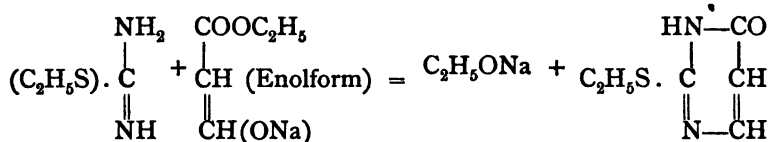
Uracil has also been prepared synthetically by Wheeler and Merriam.

Cytosine.—The knowledge of the constitution of cytosine is also due to the researches of Kossel and Steudel (*loc. cit.*). They showed that it is converted by nitrous acid into uracil. Knowing, then, the formula for uracil, there remain only two possible formulæ for cytosine, viz.,

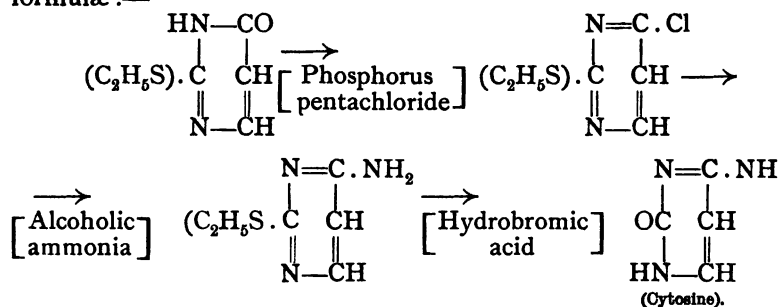


Now if formula 1 be correct, we should expect to obtain guanidine as an oxidation product ; this product is not, however, obtained when cytosine is oxidised with barium permanganate. Kossel and Steudel concluded, therefore, that formula 2 represents the constitution of cytosine. This conclusion is correct, for Wheeler and Johnson (12) have succeeded in synthesising a body of this formula, and shown that their synthetical product is identical with cytosine.

They treated ethyl-pseudo-thiurea with ethyl sodio-formylacetate, and obtained thereby 2-ethylmercapto, 6-oxypyrimidine—



This body on treatment with phosphorus pentachloride gives a chloro-derivative ; the chlorine is replaced by the amino-group by means of alcoholic ammonia.* On hydrolysing this amino-derivative with hydrobromic acid solution, the mercaptan group is readily eliminated, and the product thereby obtained is cytosine. The reactions can be represented by the following formulæ :—



In addition to the three pyrimidine bases described above Osborne and Harris (9) found a fourth amongst the degradation products of triticonucleic acid (see next lecture), which they did

* Compare these reactions with those employed by E. Fischer for synthesis in the purine series.

102 DEGRADATION PRODUCTS OF NUCLEIC ACIDS [LECT. V.]

not succeed in isolating in a pure state. With this exception, it will be seen that all the ultimate degradation products of nucleic acid appear to have been isolated and their constitutions determined.

We have, therefore, sufficient data available to be able to form some sort of a conception as to the chemical constitution of nucleic acid.

REFERENCES—LECTURE V

- (1) ASCOLI.—*H.Z.*, 31, p. 1467, 1902.
- (2) FISCHER.—*Ber.*, 32, p. 435, 1899.
- (3) FISCHER and ROEDER.—*Ber.*, 34, p. 3751, 1901.
- (4) HAMMERSTEN.—*H.Z.*, 19, p. 19, 1894.
- (5) KOSSEL.—*Ber.*, 26, p. 2753, 1893; and, 27, 2215, 1894.
- (6) KOSSEL and STEUDEL.—*H.Z.*, 37, p. 377, 1902; and, 38, p. 49, 1903.
- (7) LEVENE.—Several papers, *H.Z.*, 1902 and 1903.
- (8) NEUBERG.—*Ber.*, 35, p. 1467, 1902.
- (9) OSBORNE and HARRIS.—*H.Z.*, 36, p. 85, 1902.
- (10) SALOMON and KRUEGER.—Several papers, *H.Z.*, 1894, and onwards; also, *Ber.*
- (11) STEUDEL.—*H.Z.*, 32, p. 241, 1900.
- (12) WHEELER and JOHNSON.—*Amer. Chem. Jour.*, 29, p. 505, 1903.

LECTURE VI

THE CONSTITUTION OF THE NUCLEIC ACIDS. THE GLYCO- PROTEIDS

CONSTITUTION OF THE NUCLEIC ACIDS (*continued*)

WE now come to consider the method by which the various groups obtained by hydrolysis are combined in the molecule of nucleic acids.

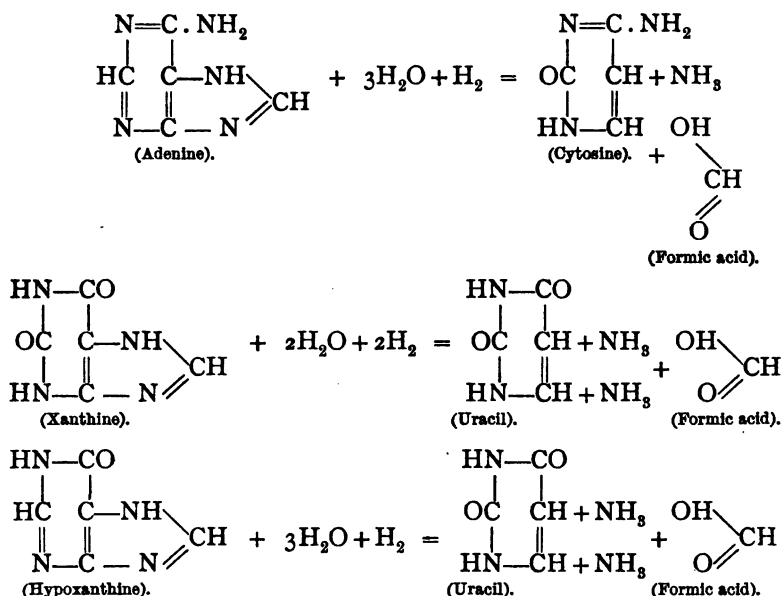
With this question is bound up another important question, viz., In how far are the various classes of degradation products described above of secondary nature? Is it possible, for example, that the pyrimidine derivatives are derivable from purine bases, and are obtained from the latter by the action of hydrolysing reagents and higher temperatures, just in the same way as lævulinic acid is derivable from a carbohydrate, or do they pre-exist as such in the nucleic acid molecule?

Are the Pyrimidine Bases Secondary Products?

Burian,* in his monograph on the chemistry of the spermatozoa, calls attention to the relationship between the pyrimidine and the purine bases that have been obtained from nucleic acid. We know that sulphuric acid in the presence of organic matter is capable of acting as a reducing agent, especially at higher temperatures, and Burian suggests that the pyrimidine bases

* *Ergebnisse der Physiologie (Biochem. Abthlg.)*, vol. iii.
108

can be directly derived from the purine bases in accordance with the following formulæ :—



There are various facts existing in favour of the above hypothesis. Kossel and Neumann have shown (see p. 97) that if the thymo-nucleic acid be treated with 30 per cent. sulphuric acid at 150°, the adenine, the scission of which readily takes place on gentle hydrolysis, is entirely destroyed. Furthermore, ammonia and formic acid are both products of nucleic acid degradation. These facts certainly support the hypothesis that the pyrimidine bases are only secondary decomposition products.

On the other hand, there are various facts which seem to negative the above hypothesis. Osborne and Harris have shown that on gentle hydrolysis of tritico-nucleic acid, obtained from wheat-meal, the whole quantity of the purine bases obtainable is eliminated in a very short time. On hydrolysing this nucleic acid with dilute acid (1 per cent. hydrochloric acid), the scission of all the purine bases obtainable was complete in

half an hour. If the residue after scission be heated with stronger acids at higher temperatures (20 per cent. sulphuric acid for two hours at 150-160°), elimination of uracil takes place. Now, the purine bases isolated from tritico-nucleic acid are guanine and adenine. Osborne and Harris call attention to the fact that the same products have been obtained by the hydrolysis of thymo-nucleic acid; from the latter acid, however, the chief pyrimidine base obtainable on more stringent hydrolysis is not uracil, but thymine, even though both tritico-nucleic acid and thymo-nucleic acid yield on gentle hydrolysis the same purine bases. Furthermore, there appears, according to Osborne and Harris's careful work, to be a quantitative relationship between the various bases eliminated from tritico-nucleic acid. They obtained very approximately one molecule of guanine and one molecule of adenine to two molecules of uracil; these facts strongly support the hypothesis that the last-named body pre-exists as such in the nucleic acid, and is not a secondary product derivable from the guanine and adenine. Furthermore, Kossel and Steudel did not succeed in preparing pyrimidine bases from purine bases by heating the latter with strong acid to 150°, although Burian believes he has isolated a cytosine-like body, when he modified the conditions of Kossel and Steudel's experiments by the addition of grape-sugar to the sulphuric acid.

On the whole, the evidence seems to favour the hypothesis that the pyrimidine bases exist as such in the nucleic acid molecule, and are not secondary products derived from the purine derivatives.

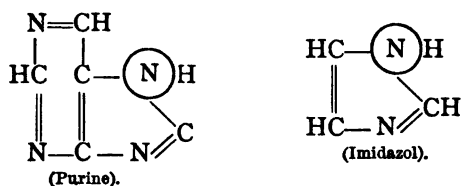
In what Relation do the Bases Stand to the Phosphorus in the Molecule?

It might have been thought originally that the bases were combined with phosphoric acid as salts. In an early stage of the researches on nucleic acid this hypothesis was shown to be untenable, for although the purine bases can be very readily eliminated by gentle hydrolysis, it was shown by Kossel and Neumann that they could not be obtained by simple extraction;

they existed, therefore, in a state of organic combination in the molecule.

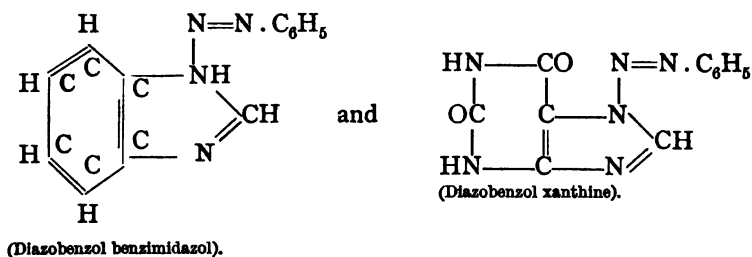
We have, then, to inquire, In what form of combination does the phosphoric acid exist? Are the bases combined with the phosphoric acid as esters, *i.e.*, through the oxygen, or are they united to the phosphorus by a nitrogen in a phosphamido form? This question has been discussed by Burian, who brings forward certain weighty arguments in favour of the latter hypothesis.

He calls attention to the fact that purine and several of the purine bases are imidazol derivatives, as will be evident from the two appended formulæ :—



Now Burian points out that all imidazol derivatives in which the nitrogen denoted in the above formulæ by a ring is still combined with hydrogen, and the latter element is *not* substituted, react with diazo-bodies to give characteristic pigments.

From benzyimidazol and xanthine diazo-derivatives of the following constitution have been prepared :—

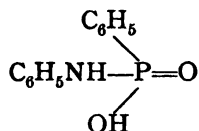


Wherever this particular nitrogen indicated above by the ring is combined, not with hydrogen but with another group,

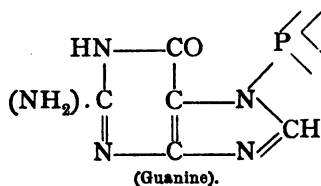
like the methyl group, as is the case with certain purine bodies like caffeine or theobromine, this reaction with diazobenzenes cannot take place.

Burian has shown that nucleic acids themselves do not give pigments with diazobenzene derivatives unless previously mixed with bodies like xanthine, which are capable of reacting. If, however, the nucleic acid be gently hydrolysed, the products of hydrolysis yield the reaction. He concludes, therefore, that xanthine is united in the nucleic acid molecule directly through the nitrogen atom.

Furthermore, Osborne and Harris have drawn attention to the fact that although scission of purine bases takes place very readily when hydrolysis is carried out by even dilute acids, this is not the case when alkalis are employed instead. This favours the hypothesis that the purine bases are combined with phosphorus directly through the nitrogen; that is to say, they are phosphamido-derivatives and not phosphoric esters. A body of the former type, such as anilino-phenyl-phosphoric acid,

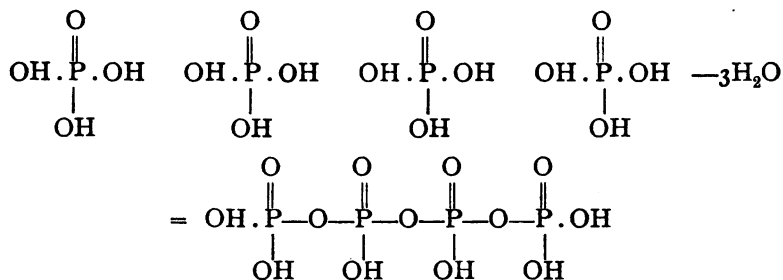


prepared originally by Michaelis, is extremely resistant to hydrolysis by alkalis; on the other hand, scission of aniline readily takes place on hydrolysis by acids. These facts seem to support the hypothesis that the purine bases are united through a nitrogen atom to phosphorus according to a scheme like the following :—

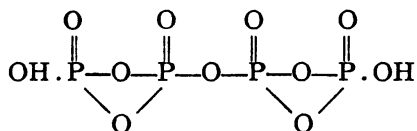


The Form of Phosphoric Acid in Nucleic Acids.

On hydrolysis, phosphoric acid is obtained apparently in the form of a polyphosphate. If we consider the constitution of phosphoric acid, we shall see at once the possibility of the condensation of several molecules of the orthophosphoric acid to form a polyphosphoric acid of great complexity, analogous in many respects to a polysilicate. A tetraphosphate, *e.g.*, is theoretically conceivable as derived from phosphoric acid, according to the following scheme:—



or even an anhydride of the form

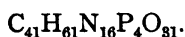


by the elimination from the above of two molecules of water.

It is obvious that we cannot obtain the phosphoric acid directly in its original form by hydrolysis, as the complex polyphosphoric acid can readily degrade into a simpler phosphoric acid. In order, therefore, to determine the constitution of a nucleic acid, it is necessary to obtain quantitatively the products of hydrolysis, and to determine, moreover, the number of hydroxyl groups pre-existing in the molecule before degradation. As an interesting example of an attempt to determine the constitution of a nucleic acid, it will be worth while to consider in some detail the researches of Osborne and Harris on the tritico-nucleic acid obtained from wheat-meal.

Osborne and Harris's Researches on Tritico-nucleic Acid.

Osborne and Harris made several preparations by varying methods, of this acid from fat-free wheat-meal. The latter, obtained in a fine state of subdivision by pressing through cloth, was extracted with large quantities of cold water in a cool place. The turbid extract was syphoned off from the precipitate, and saturated with sodium chloride, and to this mixture acetic acid was added. A nucleine was thereby precipitated, from which the nucleic acid was obtained by peptic digestion. Various methods were employed, such as Levene's picric-acid method (see p. 76), etc., for separating the products of albumen origin from the acid, which was finally obtained in the form of an acid potassium or sodium salt by precipitation of the neutral solution of the potassium or sodium salt in water by alcohol containing excess of hydrochloric acid. Products were obtained in different preparations containing varying amounts of sodium or potassium. From the analyses of these salts, the empirical formulæ of the free acid were calculated: as the result of the analyses of those various preparations, Osborne and Harris assigned to the tritico-nucleic acid the following formula:—



This can hardly be more than approximate.

They then determined the following factors:—

(a) *The number of Hydroxyl Groups.*—This was done by preparing and analysing the silver salts. They obtained a salt containing silver to correspond with the formula



They concluded, therefore, that the nucleic acid contained six hydroxyl groups, and was therefore hexa-basic.

(b) *The Carbohydrate Groups.*—These were determined quantitatively, by distilling weighed portions of the nucleic acid for seven hours with 12 per cent. hydrochloric acid. Furfural was evolved, and this was quantitatively estimated by preparing the phloro-glucide.

(c) *The Purine Bases.*—These were estimated by the method

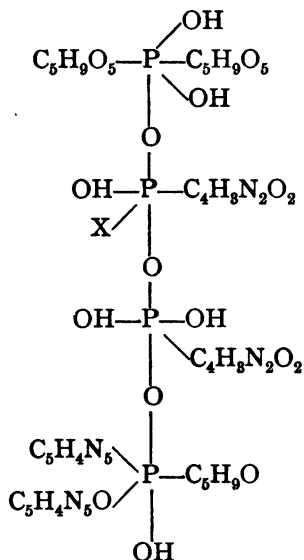
of Kossel already described: they were readily isolated after gentle hydrolysis (*vide supra*, p. 97).

(d) *The Pyrimidine Bases*.—Amongst these was found uracil, and another base which was not obtained in a pure state, but which gave an insoluble barium salt, when the phosphotungstic acid precipitate of the base was decomposed by baryta.

From the result of the various quantitative determinations, Osborne and Harris concluded that tritico-nucleic acid contains the following groups and atoms:—

1 molecule Guanine ($C_5H_5N_5O$)	3 molecules Pentose ($C_5H_{10}O_5$)
1 molecule Adenine ($C_5H_5N_5$)	6 Hydroxyl groups
2 molecules Uracil ($C_4H_4N_2O_2$)	4 atoms Phosphorus
3 atoms Oxygen in combination in a form other than hydroxyl, and possibly coupling the four phosphorus atoms.	

These constituents would make up 87.8 per cent. of the whole molecule. They found in addition another base (X), which they were not able to identify. They suggest for tritico-nucleic acid the following formula:—



Such a formula as the one suggested can only be approximate. It gives a good idea, however, of the possible complexity of a nucleic acid molecule, especially when we attempt to substitute the rational for the empirical formulæ of the various groups. When we remember, moreover, that in nature some of these hydroxyl groups are combined with basic groups of an albumen, the general complexity of a nucleine may be readily understood.

The Nucleo-albumens and Para-nucleines.

Nucleo-proteids on peptic digestion are hydrolysed. The albumen part of the molecule gives the usual products of peptic digestion, and a nucleine remains undissolved; this, on treatment by the methods already described, yields a nucleic acid, from which on hydrolysis purine and pyrimidine bases can be obtained.

There is, however, another class of bodies, which were at first mistaken by Miescher for nucleo-proteids, and which, like the latter, are also rich in phosphorus; on peptic digestion they also yield a residue richer in phosphorus than the original substance; they differ very markedly, however, from the nucleine residue obtained in a similar way from nucleo-proteids, in that they do not on hydrolysis yield purine bases; chemically they are quite distinct, therefore, from true nucleines. The bodies yielding these phosphorus-rich residues on tryptic digestion have been called by Kossel and Hammersten the nucleo-albumens, in contradistinction to the term nucleo-proteids, the bodies containing the now well-defined nucleic acid as a prosthetic group. The residue obtained from the nucleo-albumens on peptic digestion is called *para-nucleine*, or *pseudo-nucleine*.

The phosphoric acid appears to resist scission when treated with acids, although it is readily eliminated from the molecule by alkalis.

The two chief examples of albumens belonging to this group are caseine and vitelline; included in it is a series of cell nucleo-albumens. The bodies of this class are generally fairly strong acids, the salts of which are soluble in water; from solutions of the salts the free bodies can be precipitated by acids. The

solutions of the salts are not as a rule coagulable, and can generally be heated without change. They can be readily salted out of solution—in fact, more readily than the globulins; in this respect they resemble fibrinogen and myosine.

They are more readily attacked by alkalis than the true nucleines; this suggests that they are possibly esters of phosphoric acid. We know but little yet of their degradation products, so that not much can be said as to their constitution.

THE GLYCO-PROTEIDS.

We proceed now to discuss the second class of bodies containing a prosthetic group, viz., the glyco-proteids, bodies from which on hydrolysis a carbohydrate group is readily obtained.

These must be distinguished, in the first place, from ordinary albumens, from several of which carbohydrate groups have been isolated, in that the latter require somewhat drastic hydrolysis before the sugar group, or the parent substance of the sugar group, is eliminated. The glyco-proteids are widely distributed; the principal substances belonging to this class are the mucines, mucoids, and cartilage.

Our knowledge of the carbohydrate groups in these products is still far from satisfactory. They have formed the subject of a large number of researches by Schmiedeberg and his pupils. Although many gaps remain to be filled up in the researches on the subject, and more modern methods may with advantage be applied, the researches of Schmiedeberg must still be regarded as the standard work on this subject. It will be advisable, then, to consider in some little detail his researches on cartilage. (*Schmiedeberg's Researches on Cartilage* (8); commenced in 1883; published, 1891.)

A reducing substance from cartilage had been obtained as long ago as 1861, by Boedecker, to which the name chondroitin acid was given. It formed the subject of many subsequent researches, amongst which may be mentioned those of Krukenberg and Mörner.

Schmiedeberg's research was undertaken with the object of isolating the parent substance, which on hydrolysis yields sugar. The product obtained by him was called chondroïtine; to it the empirical formula $C_{12}H_{21}NO_{11}$ was assigned.

The method of preparation employed by Schmiedeberg was the following:—The prepared nasal cartilages from a large number of animals (pigs—not, as a rule, more than sixty or seventy at a time, however), were soaked in water for twenty-four hours, to free them from blood, dirt, etc. The wash-water was repeatedly renewed during this time. The cleaned cartilages were then pressed through a mincer, and digested with a strong preparation of pepsine prepared from pigs-stomach in 0.3 per cent. hydrochloric acid solution.

After twenty-four hours digestion at $36-37^{\circ}$, and addition of water, a pasty mass separated out; this is a compound of chondroïtine sulphuric acid with peptone, and is called by Schmiedeberg *pepto-chondrine*. It is often obtained mixed with a corresponding gluten compound called gluten-chondrine.

The pepto-chondrine is purified by dissolving it in 2-3 per cent. hydrochloric acid, in which it is readily soluble; on treatment of this solution with alcohol, pepto-chondrine is precipitated; it is obtained thereby in a purer state, as by dissolving in hydrochloric acid it is separated from undigested cartilage, nucleine bodies, gelatine, etc.

The next stage in the process consists in separating the sugar-yielding prosthetic group from the albumenous groups, and for this purpose Schmiedeberg employs a process analogous to the one suggested by him for preparing nucleic acid from nucleines (see p. 75); the prosthetic group is obtained in the form of the potassium copper salt, and thereby separated from the bodies giving the biuret reaction.

For this purpose the crude pepto-chondrine is dissolved in alkali; copper acetate and potash are then added to this solution alternately, until the liquid assumes a deep violet colour with a blue opalescence. Alcohol is then added, and a cupric-potassium salt is thereby precipitated; the precipitant should be added in such quantity that the supernatant liquid

H

has a pure violet colour, and no longer opalesces. By means of this treatment the greater part of the albuminous group is removed in the form of the body which yields a biuret reaction with alkaline copper salts.

The copper-potassium salt is then redissolved in water containing potash and reprecipitated by alcohol, and this process of reprecipitation is repeated until the supernatant liquid no longer shows a violet colour.

Even now, the product is not quite free from foreign bodies ; it is still apt to contain a little nucleic acid as impurity ; to free it from this, the alkaline solution of the copper-potassium salt is acidified, and alcohol is added, until a permanent precipitate just forms. This is filtered off, the filtrate is made alkaline again, and alcohol is added to precipitate the remainder.

This is purified by re-solution in water, and reprecipitation after exact neutralisation of the solution, with hydrochloric acid by alcohol.

In this way a sulphuric acid ester is obtained, the so-called chondroitine sulphuric acid. It is an amorphous body, which has not yet been obtained in a pure state, owing to the ease with which it decomposes, and the difficulty of obtaining it water-free by drying in vacuum at ordinary temperatures. Its salts, furthermore, are extremely difficult to prepare in a pure state, owing to the tendency to form acid or basic compounds with metals.

As the result of the analyses of a large number of (only at the best approximately) pure preparations of different salts, Schmiedeberg assigns to chondroitine-sulphuric acid the formula $C_{18}H_{27}NSO_{17}$.

Scission of sulphuric acid takes place when the free chondroitine-sulphuric acid is treated with hydrochloric acid. For the purpose of this reaction, the free chondroitine-sulphuric acid is best prepared from its barium salt by treating the latter with sulphuric acid. It is precipitated from its solution by a mixture of alcohol and ether. The precipitate is allowed to stand for several days in a warm place in aqueous solution containing free hydrochloric acid. The scission is nearly completed by heating the mixture to a higher temperature. This

last reaction requires considerable care, as the product readily undergoes degradation on boiling with acids. On adding alcohol, a product is precipitated which still contains sulphur. This is again allowed to stand for several days in a warm place, in aqueous solution containing hydrochloric acid, and again reprecipitated with alcohol. The process is repeated until finally a product is obtained which is perfectly free from sulphur.

The substance obtained by this tedious process is the so-called chondroitine. In a pure state, it should be perfectly free from reducing substance and also free from sulphur. It is, however, extremely difficult to prepare in this state of purity. To it the following formula has been assigned—

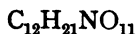


From this product which is a mono-basic acid, by hydrolysis with acids bodies can be obtained which are capable of reducing alkaline copper solutions.

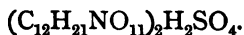
The first degradation product produced by hydrolysis which Schmiedeberg isolated was the so-called *chondrosine*. This body can be isolated in the form of a sulphate.

For its preparation a mixture of chondroitine and chondroitine sulphuric acid serves. The hydrolysis is performed most conveniently by a 2-4 per cent. solution of nitric acid on a water-bath. On addition of alcohol and ether to the solution after sufficient concentration, chondrosine is precipitated in the form of its sulphate.

The free body has the formula



and its sulphate the formula

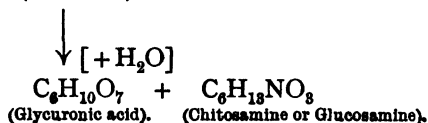
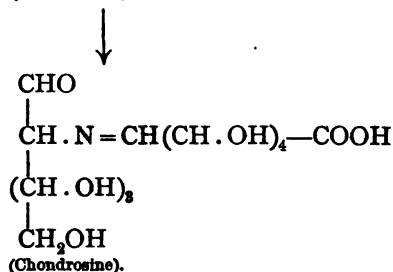
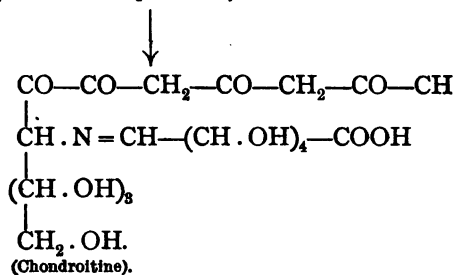
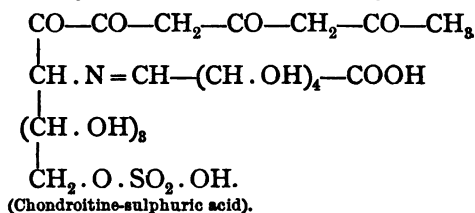


In spite of the fact that chondrosine forms salts with acids, it is itself an acid. It forms salts both with acids and bases, like bodies containing both the amino- and carboxyl-groups. Its metallic salts are mostly soluble in water; it readily reduces alkaline copper solutions; it is dextro-rotatory.

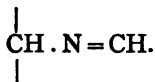
On treatment with baryta, chondrosine readily degrades into simpler products.

With baryta at 40°-50°, Schmiedeberg thought he had obtained glycuronic acid. On boiling for a long time with baryta, three different nitrogen-free acids were obtained, and the solution gradually lost its copper-reducing power.

Schmiedeberg also assumed that glycosamine was produced simultaneously with glycuronic acid. He did not succeed, however, in obtaining any of the simple degradation products of chondrosine in a pure state. His scheme of formulæ to represent the different reactions is the following :—

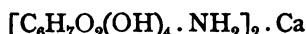


It has been remarked already that Schmiedeberg did not isolate his final degradation products in a state of purity, and the formulæ suggested above are therefore open to doubt. Attention must be called here to the fact that chondrosine contains the group



This would hardly be sufficiently basic to produce a sulphate which would be stable in water.

Furthermore, more recent work has thrown doubt on the conclusions drawn by Schmiedeberg as to the formation of glycuronic acid and an amino-sugar. Orgler and Neuberg (5) have recently undertaken a fresh investigation of chondrosine. They hydrolysed this product with baryta, and fractionally precipitated the scission products with lead acetate. A fraction was obtained from which a crystalline copper salt was prepared. This had the following formula :—



It is, *i.e.*, the copper salt of a hydroxy-amino acid. Orgler and Neuberg doubt whether glycuronic acid or glycosamine are really formed. The acid of which they isolated the copper salt is dextro-rotatory; its constitution is not yet known; the products of hydrolysis of chondrosamine are still under investigation.

Although, therefore, cartilage on gentle hydrolysis yields a prosthetic group, from which by further degradation a reducing body is readily obtained, a large amount of work remains to be accomplished before our knowledge of this subject is complete.

The above short sketch is, however, sufficient to give some idea of the experimental difficulties of the investigation. The higher products are unstable, and have not been obtained in a crystalline form, and improvements must be introduced into our technique for dealing with them before we can arrive at any satisfactory conclusion as to their true nature.

The Mucines and Mucoids.

In 1852 Scherer (7) observed that a reducing body was produced when ovarian liquid was submitted to hydrolysis by means of acids. The albumen body from which the reducing body was prepared was designated metalbumen.

Hammersten (2) showed later that this body, which produces sugar on hydrolysis, is closely related to the mucines.

These latter products have, since the work of Scherer and Hammersten, been the subject of a large number of investigations.

An intermediary complex body was obtained in many cases which only on drastic hydrolysis with acids yielded a copper-reducing body. Many controversies arose as to whether such bodies were in reality derived from the proteid itself, or from glycogen-like substances with which the proteid was supposed to be mixed. Landwehr assumed that such substances existed, and designated them "animal gums."

Hammersten and Landwehr on treatment of mucins with alkali (in the cold) obtained a polymerised carbohydrate, which only on hydrolysis with acids yielded the copper-reducing body. Hammersten maintained, in opposition to Landwehr, that the "animal gum" was a direct degradation product of the proteid. The former obtained a nitrogenous-reducing body on treatment with alkalis, and found that the amount of nitrogen varied with the strength of alkali used. He maintained, therefore, that the "animal gum" was a degradation product of the proteid, and not, as Landwehr assumed, a simple admixture.

The view of Hammersten as to the glyco-proteid nature of the mucines is now generally adopted. For our present knowledge of these bodies we are indebted chiefly to the researches of Schmiedeberg and Friedrich Müller and their pupils.

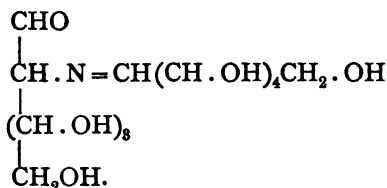
Leathes (4) in Schmiedeberg's laboratory investigated the scission products of paramucine, a body which forms a gelatinous mass in certain ovarian cysts. This body in alkaline solution reduces Fehling's solution without any further treatment.

For the isolation of the carbohydrate part of the molecule,

Leathes used a process similar to that employed by Schmiedeberg for the preparation of chondroitine-sulphuric acid. The paramucine was digested with pepsine in hydrochloric acid solution. Copper acetate and potash were then added, and a violet colouration was produced in the liquid, and a light-blue gelatinous copper salt precipitated. The latter can be freed from the biuret bodies by dissolving in acids and reprecipitating by alkalis. When the supernatant liquid is quite free from the violet biuret colouration, the copper-potassium salt is dissolved in acids (acetic or hydrochloric), and alcohol is added to the solution thus prepared. An ash-free white hygroscopic powder is thereby obtained. This consists of a body (not yet in a state of perfect purity) to which Leathes assigned the formula



He assigned to it the following constitutional formula—



This body on treatment with acids yields copper-reducing substances. An osazone of an amino-sugar was obtained from the products of hydrolysis; this sugar Leathes assumed to be glycosamine, a surmise which Steudel has subsequently proved to be correct. The other scission product of paramucine is probably gulose, a sugar obtainable by the reduction of glycuronic acid.

The other chief researches on the mucine sugars are those of Friedrich Müller* and his pupils. Using the method, which we have already discussed, of Baumann-Schotten, they have isolated from the decomposition products of the mucines glycosamine in the form of its benzoyl derivative.

Glycosamine, therefore, can be obtained as a degradation

* Cf. Carbohydrate groups of Albumens (Lecture II.).

product from a large number of albumens and glyco-proteids ; in the latter bodies it appears to be derived from a prosthetic group. It must be again remarked, however, that much remains to be done before our knowledge of carbohydrate groups both in albumens and in glyco-proteids can be considered satisfactory.

REFERENCES—LECTURE VI

- (1) BURIAN.—*Ergebnisse, loc. cit.*, vol. iii.
- (2) HAMMERSTEN.—*Arch. f. d. ges. Physiol.*, 36, 373 ; *H.Z.*, 12, p. 163, 1888.
- (3) LANDWEHR.—*H.Z.*, 5, p. 371, 1881 ; *H.Z.*, 6, p. 75, 1882.
- (4) LEATHES.—*A.P.P.*, 43, p. 245, 1899.
- (5) ORGLER and NEUBERG.—*H.Z.*, 37, 407, 1902.
- (6) OSBORNE and HARRIS.—*H.Z.*, 36, p. 85, 1902.
- (7) SCHERER.—*Chem. u. Mikrosk. Untersuchungen*, Heidelberg, 1843.
- (8) SCHMIEDEBERG.—*A.P.P.*, 28, p. 355, 1891.

LECTURE VII

THE CHEMISTRY OF HÆMOGLOBIN AND ITS CHROMATOGENIC GROUP

As examples of the proteids, we gave in Lecture II. three instances, viz., the nucleo-proteids, the glyco-proteids, and hæmoglobin; the two former yield as their prosthetic groups nucleic acid and a carbohydrate group; the last-named yields a chromatogenic group.

Hæmoglobin is an example of an albumen which can be easily obtained in a crystalline form; as might be expected from its physiological importance, it has been the subject of very numerous chemical researches. It forms compounds with oxygen, carbon-monoxide, and other bodies; we shall not, however, concern ourselves with these products here, but proceed immediately to the consideration of the degradation products, especially those of the interesting and important chromatogenic group.

Preparation of Crystallised Hæmoglobin (Hoppe-Seyler).

As hæmoglobin is more readily soluble than the oxy-body, the latter is better adapted for the preparation of a crystalline compound.

Hoppe-Seyler's method for preparing crystallised oxyhæmoglobin is as follows:—The washed blood-corpuscles (of a horse or dog) are stirred with twice their volume of water, and the mixture is then shaken with ether, which serves to break the envelopes of the corpuscles. After separation of the ether, the

dark-coloured solution which is obtained is allowed to stand in an open basin; the ether remaining in aqueous solution evaporates. The ether-free solution is then filtered and cooled to 0° C.; alcohol, cooled previously to 0° , is then slowly added, the mixture being kept continually stirred during the addition. When the alcohol added amounts to about 25 per cent. of the volume of the original aqueous solution, the mixture is cooled to between -5° and -10° , and allowed to stand for some days at this temperature. The oxyhæmoglobin then separates in crystalline form. It can be recrystallised by dissolving it in water at 35° , cooling this solution to 0° , adding cold alcohol gradually and with constant stirring (1 vol. alcohol to 4 vols. aqueous solution), and again allowing the mixture to stand for some days at a temperature of -5° to -10° C. The crystals, after a sufficient number of recrystallisations, are finally washed with cold alcohol and water (1 vol. alcohol, 4 vols. water), and dried in vacuo at 0° .

Hæmoglobin gives amongst its immediate products of decomposition very small quantities of volatile fatty acids, an albumen (globine), and an iron compound (hæmochromogen or hæmatine, according to the conditions of decomposition; the former body is produced in the absence of air, the latter in the presence of air and acids).

Separation of the Albumen Part of the Molecule from the Chromatogenic Part.

It requires only very gentle treatment to separate the chromatogenic prosthetic group from the albumen part of the molecule.

Method of Schulz (9).—In the experiments of Schulz oxyhæmoglobin of horse-blood was employed. This was prepared in a crystalline form by allowing it to separate from a solution which had been half saturated with ammonium sulphate (compare preparation of crystallised egg-albumens). The blood was treated first with ammonium oxalate to inhibit coagulation, and the blood corpuscles were allowed to separate out. The pasty mass thus obtained was then diluted with twice the volume

of water, cooled, and to the cold solution an equal volume of saturated ammonium sulphate (also cooled to 0°) was added. The globuline and fibrinogen separate out, and are rapidly filtered off in an ice-chest. On allowing the solution of the hæmoglobin thus obtained to stand at ordinary temperatures, crystals rapidly commence to separate out, and the process of crystallisation can be completed by the addition after a time of more ammonium-sulphate solution.

By the action of very weak acid on hæmoglobin, a decomposition takes place into the albumen group and the chromatogenic group. On treating the hæmoglobin prepared by Schulz's method with small quantities of very dilute hydrochloric acid a precipitate of pigment forms, which, if the hæmoglobin used be free from inorganic salt, rapidly dissolves in excess of the acid. Acid is added until the precipitate has completely dissolved, and then alcohol and ether are added in such relative quantities (to be determined by preliminary experiment) that the ether separates rapidly from the aqueous solution. The colouring matter dissolves then in the ether, whilst the albumen (globine) remains in solution in water.

On neutralising the aqueous solution with ammonia, the globine is precipitated.

This body is soluble both in acids and alkalis; it is insoluble in ammonia, however, in the presence of ammonium chloride; hence, the above-mentioned method is employed for its precipitation from solution. It is coagulable on heating, but the coagulum is soluble in acids. It contains as much as 16.89 per cent. nitrogen. Schulz, on account of these properties, considers globine as a histone.

Lawrow's Experiments (5).

Lawrow made experiments with the object of determining quantitatively the relative amounts of prosthetic groups and albumens. He decomposed the hæmoglobin by means of a mixture of alcohol, ether, and sulphuric acid, which was added very gradually to the hæmoglobin solution. The globine was thereby precipitated, the chromatogenic group remaining in the

aqueous ethereal alcoholic solution. This latter, on evaporation of the alcohol and ether, deposits the chromatogenic group in the form of hæmatine, which is perfectly free from an albumen contamination.

Lawrow found, as the result of his experiments, that hæmoglobin yields

94.09 per cent. albumen,
4.47 per cent. hæmatine,
1.44 per cent. other constituents.

Schulz obtained 4.2 per cent. hæmatine, but smaller quantities of albumen.

It will be noticed from the results both of Schulz and Lawrow, that the chromatogenic part of the molecule is under 5 per cent. It forms, therefore, only quite a small part of the hæmoglobin molecule.

The results obtained by the degradation of globine by Fischer's method have been already referred to (see p. 62).

The Chemistry of the Chromatogenic Group.

We shall now proceed to discuss the experiments that have been carried out with the object of determining the chemical constitution of the group to which the pigmentation of hæmoglobin is due.

Hæmatine and its related Products.—As already stated, the chromatogenic group can be obtained from hæmoglobin either in the form of hæmochromogen, in the absence of oxygen, or in the form of hæmatine (or hæmine, a hydrochloric-acid derivative of hæmatine) in the presence of air.

Hæmatine has been the subject of many investigations, and considerable controversy has arisen as to whether the products obtained by different methods and from different animals are identical. The recent researches of Küster, however, point to the conclusion that only one hæmatine exists.

Preparation of Hæmine and the Researches of Küster (2).—More than fifty years ago, Teichmann observed that when a drop of blood is mixed with a drop or two of glacial acetic

acid, and the mixture is allowed to evaporate over a very small flame, a residue is obtained, which contains innumerable reddish-brown prismatic needles, known generally since the discovery as "Teichmann's crystals." In 1884 Nencki and Sieber published their researches on the preparation of the crystalline body on a larger scale, with the object of determining its constitution. Since that time, a large number of other investigators have devoted their attention to the subject, and have devised various methods for obtaining crystals of the pigment.

Nencki and Sieber in 1884 published a method for preparing hæmine, the principal of which consisted in the extraction of blood with amyl alcohol containing hydrochloric acid.

Other methods are due to Cloetta (1895), Mörner (1897), Schalféjew (1898), Rosenfeld (1898), and others.

In Mörner's method the coagulated blood was extracted with methylated spirit containing $\frac{1}{2}$ to 1 vol. per cent. concentrated sulphuric acid. The solution of the pigment was then heated to the boiling-point and hydrochloric acid was added; on cooling, crystals separated.

Of all the methods employed, however, for obtaining crystals of pigments, that due to Schalféjew gives the best yields.

For this purpose 1 volume of defibrinated and strained blood is added to 4 volumes of glacial acetic acid previously heated to 80° C. As soon as the temperature has fallen to 55°-60°, the liquid is again warmed to 80°; on cooling, crystals at once separate. These are allowed to settle for at least twelve hours, and the clear dark-brown mother-liquor is then syphoned off. The crystals are then washed by decantation with water, thrown on a filter, and then washed again, first with distilled water, then graded strengths of alcohol, commencing with the more dilute, and finally with alcohol and ether.

Rosenfeld extracted the alcohol-coagulated blood with an alcoholic solution of oxalic acid; from the solution thus obtained the pigment was precipitated with hydrochloric acid.

To the hæmine prepared by the Nencki-Sieber method, was ascribed the formula



To the hæmine prepared by the Schalféjew method, Nencki and Zaleski ascribed the formula



This was supposed to be an acetyl derivative, and was known as acethæmine.

Finally, Mörner ascribed to the body prepared by his method the formula



This was called β -hæmine.

Küster has examined the products prepared by four different methods, viz., those of Nencki and Sieber, Schalféjew, Mörner, and Rosenfeld, and has come to the conclusion that they are all identical. He employed a method for purification in all cases, that was due originally to Schalféjew, and which is carried out as follows:—1 gram of the crude material is shaken for fifteen minutes with 40 c.c. of chloroform in which 5 c.c. of pyridine have been dissolved. The solution is filtered and then thrown into 150 c.c. of glacial acetic acid previously saturated with sodium chloride, and heated to 105° C.; 0.5 c.c. of concentrated hydrochloric acid are then added, and the mixture is allowed to stand for twenty-four hours; the crystals which then separate out are filtered off, washed with dilute acetic acid containing hydrochloric acid, and then dried.

Quinine can be used instead of pyridine in the above method.

Küster found that hæmines prepared by the various methods described above (methods of Nencki and Zaleski, Mörner, Rosenfeld, etc.) do not entirely dissolve in the chloroform containing pyridine or quinine. This residue was termed by Schalféjew the "corpses" ("carcasses"). Küster prepared hæmines by the methods of Nencki, Mörner, and Rosenfeld. The various preparations were all purified by the Schalféjew

method. The products obtained by these methods were all analysed. As a result, Küster comes to the conclusion that only one hæmine exists, as the analysis numbers of the various preparations were all identical. He ascribed to hæmine the formula



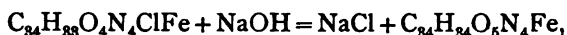
Identical analysis numbers were also obtained when the preparations were made from ox-blood and horse-blood.

The purification by means of chloroform containing pyridine or quinine is not apparently a recrystallisation in the ordinary sense of the word; scission and readdition of hydrochloric acid appears to take place during the process.*

Hæmatine.—By suitable treatment of hæmine with alkalis, hæmatine can be produced. The hæmine is generally dissolved in alkalis (caustic soda or ammonia), and the alkaline solution is then thrown into excess of acids. These processes are generally repeated several times. The formula of hæmatine has not yet been determined with certainty. It is probably either



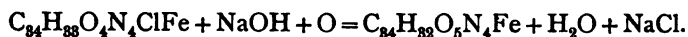
which can be derived from hæmine according to the equation



or



derived according to the equation

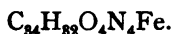


It is almost certain that hæmatine is produced from hæmine by the replacement of chlorine by a hydroxyl group; the former is not merely a hydrochloride of the latter.

Dehydrochloride of hæmine.—It has been stated above that in the Schalféjew method of purifying hæmine by treatment with pyridine or quinine in chloroform solution, a scission of hydrochloric acid takes place; on pouring the basic chloroform solution into acetic acid containing hydrochloric acid, readdition

* The general results of Küster have since been confirmed by Hetper and Marchlewski.

takes place. Küster has succeeded in isolating the intermediary product (by the action of aniline), dehydrochloride of hæmine, and re-synthesising from this body hæmine. The dehydrochloride has the formula



Hæmatoporphyrine.—Another body related to hæmine and hæmatine is the so-called hæmatoporphyrin; the investigations on this body have led to some important results bearing on the general chemistry of the blood-pigments; and it will be necessary to consider its relationship to hæmine and hæmatine, of which it is an iron-free derivative.

Nencki and Zaleski prepared hæmatoporphyrin in the following way:—5 grams of hæmine (it is advisable to work with small quantities at a time) are added gradually in small portions to a saturated solution of hydrobromic acid in glacial acetic acid, maintained at a temperature of about 10° C. The mixture is vigorously shaken after each addition of the solid. The whole is then allowed to stand at room-temperature for three or four days, and repeatedly shaken until the whole of the hæmine has passed into solution, which latter attains a beautiful bright red colour. The mixture is then thrown into distilled water, and from this diluted solution after several hours a small quantity of solid separates. This is filtered off, and caustic soda is then added to the filtrate in quantities just sufficient to neutralise the hydrobromic acid. By this means the hæmatoporphyrin is almost completely precipitated. The precipitate is washed by decantation until it is quite free from bromide. It is then freed from the greater part of the adhering liquid by blotting-paper. Whilst still moist, it is warmed on the water-bath with dilute pure caustic soda; a solution is formed, together with a precipitate of oxide of iron; after filtration, acetic acid is added to the liquid, whereby the hæmatoporphyrin is reprecipitated.

This body forms a hydrochloride which is readily soluble in water, but less readily soluble in hydrochloric-acid solution; from the latter it can best be recrystallised.

The formula of hæmatoporphyrin has been established by careful analyses by Zaleski (11), who not only analysed hæmatoporphyrin itself, but also another product, viz., mesoporphyrin, which we shall have to discuss shortly, and which he showed could be obtained directly from hæmatoporphyrin by reduction with hydriodic acid and phosphonium iodide. Zaleski prepared various salts and esters of both mesoporphyrin and hæmatoporphyrin; he also determined the molecular weight of the former, as well as that of its ethyl ether; as the result of his researches, he assigned the following formulæ to various bodies he investigated:—

$C_{84}H_{88}O_4N_4 \cdot 2HCl$	-	Mesoporphyrin hydrochloride.
$C_{84}H_{88}O_6N_4 \cdot 2HCl$	-	Hæmatoporphyrin hydrochloride.
$C_{84}H_{86}O_4N_4(C_2H_5)_2$	-	Ethyl ether of mesoporphyrin.
$C_{84}H_{86}O_4N_4Zn$	- -	Zinc salt of mesoporphyrin.
$C_{84}H_{88}O_4N_4$	- - -	Free mesoporphyrin.

He concludes that the formation of hæmatoporphyrin from hæmine may be represented by the following equation—



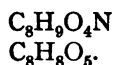
The formula $C_{84}H_{88}O_6N_4$ is assigned to hæmatoporphyrin rather than the formula $C_{17}H_{19}O_3N_2$, on the ground that a solution of mesoporphyrin in phenol caused a depression of freezing point corresponding to the molecular weight 486.4. The molecular weight of a body of the formula $C_{17}H_{19}O_3N_2$ is 282, *i.e.*, but little more than half the number obtained by experiment.

The Degradation Products of Hæmine, Hæmatoporphyrin, etc.

Our knowledge on this subject is due to the researches of several investigators. Schunck and Marchlewski (10) studied various derivatives of chlorophyll, and noticed the relationship both in the empirical formulæ, and in the absorption spectra to bodies obtained from blood-pigments. Nencki and Zaleski (8a) attempted to obtain from hæmine some chlorophyll derivatives; Küster studied directly the oxidation products of hæmine,

hæmatine, and hæmoporphyrin. We shall proceed to discuss the relationship of these various researches—undertaken from different standpoints, and independently—to one another.

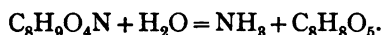
Küster's Researches (3).—Küster oxidised hæmatine with sodium bichromate and acetic acid, using the reagents in such proportions that eight atoms of oxygen were available for one molecule hæmatine. He obtained as main products two acids, viz.,



These bodies he called the hæmatinic acids.

Under favourable conditions the former acid is obtained in yields of 50 per cent. of the hæmine. These acids were obtained from the hæmines (or hæmatines) of all the bloods investigated. As, together with these acids, a product was obtained containing iron, Küster concluded that the acid is formed only from that part of the molecule to which the iron is *not* attached.

The acid $\text{C}_8\text{H}_9\text{O}_4\text{N}$ is monobasic, and yields on treatment with alkalis the second acid mentioned above, viz., the hæmatinic acid of the formula $\text{C}_8\text{H}_8\text{O}_5$ —



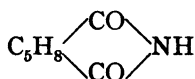
This acid $\text{C}_8\text{H}_8\text{O}_5$ is monobasic, and is the partial anhydride of a tribasic acid of the formula $\text{C}_8\text{H}_{10}\text{O}_6$, a fact which was determined by titration, and the preparation of a silver salt. The relationship of the two hæmatinic acids can be represented by the following formulæ:—



This relationship was confirmed by the fact that the nitrogen-free acid can be directly converted into the other acid by the action of alcoholic ammonia.

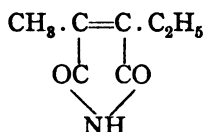
On heating the ammoniacal solution of the ammonium salt of the imido-acid to 125° – 130° C. in a sealed tube, a scission of

carbonic acid from the molecule could be brought about, and a body of the formula



obtained.

The fact that hæmine on distillation yields pyrrol, led Küster to believe that the above acid was an imide of a maleic acid anhydride; its properties, moreover, were very analogous to those of pyro-chinonic acid. He assigned to the body provisionally the formula—

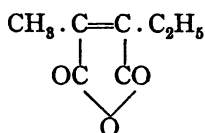


The relationship of such a body to a pyrrol derivative is at once evident.

This body on hydrolysis with baryta yielded an anhydride of the formula



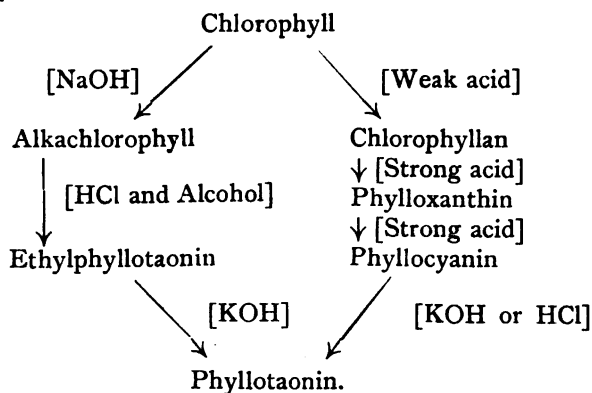
This is very similar in properties to methyl-ethyl-maleic anhydride which had been prepared by various methods (by Fittig, by Michael and Tissot, and by Bischoff). It would therefore have the probable constitution—



Hæmatoporphyrin yields the same oxidation products.

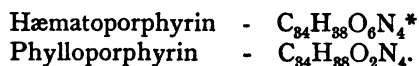
Researches of Nencki and Zaleski (8a), and Schunck and Marchlewski (10).—When the researches of Küster had reached the point above described, a further stimulus was given to investigations on the blood-pigment by some results obtained by Schunck and Marchlewski, when working at the derivatives of chlorophyll. They obtained a series of derivatives from this

plant-pigment, which may be schematically represented as follows:—



Now phyllotaonin was found to yield on treatment with alcoholic caustic soda in sealed tubes at 190° a product which was isolated in the form of a crystalline zinc salt. The body prepared from the zinc salt was found to be very similar in its properties to hæmatoporphyrin; Marchlewski somewhat later demonstrated the similarity in the absorption spectra. It was called phylloporphyrin.

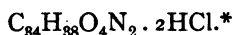
The following empirical formulæ represent the relationship of hæmatoporphyrin to phylloporphyrin—



In consequence of these researches, Nencki and Zaleski endeavoured to obtain phylloporphyrin directly from blood-pigments. They treated hæmine, or hæmatoporphyrin, with hydriodic acid dissolved in acetic acid, with the addition of phosphonium iodide; they expected to obtain a phylloporphyrin by the reducing action of the hydriodic acid. Instead, however, of this body, they obtained in the form of a microscopic

* The formulæ given in the original papers have been altered, in accordance with the results of the more recent researches of Zaleski, *H.Z.*, 37, p. 54, 1902.

crystalline hydrochloride another product, which was shown on analysis to have the formula



This body, it will be observed, is intermediate in its formula between phylloporphyrin and hæmatoporphyrin; it contains two atoms more oxygen than the former, and two less than the latter; to it Nencki and Zaleski assigned the name *mesoporphyrin*.

By the action of caustic soda on the hydrochloride, the free mesoporphyrin was obtained in the form of microscopic crystals.

If the action of hydriodic acid and phosphonium iodide be allowed to proceed more vigorously, another body which is volatile with steam is obtained as the main product. This product yields a crystalline picrate, and gives an insoluble mercury compound; it also gives with sulphuric acid and pine splinters the colour characteristic of pyrrol derivatives. It has the formula



To it Nencki and Zaleski assigned the name *hæmopyrrol*. On standing exposed to air, hæmopyrrol readily oxidises and yields a red substance; if ammonia be added to the coloured solution until the reaction is alkaline, the colour changes to yellow; on addition of an ammoniacal solution of a zinc salt, the yellow colour changes to pink, with a beautiful green fluorescence. These reactions are characteristic of the pigment urobilin, the cause of urobilinuria after blood extravasations. Furthermore, when hæmopyrrol is given to an animal *per os*, it is excreted in a very short time in the form of urobilin. Hæmopyrrol appears, therefore, to be a normal degradation product of blood-pigments in the organism.

Although phylloporphyrin was not directly obtained from hæmatoporphyrin, the intimate relationship of these derivatives of the plant-pigment chlorophyll and the blood-pigment hæmoglobin was very shortly afterwards demonstrated by Nencki and

* See preceding footnote.

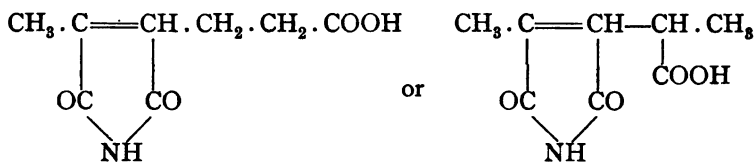
Marchlewski, who, when the researches had reached the stage just indicated, joined forces and continued the work conjointly.

They showed that phyllocyanin, from which the phylloporphyrin is prepared (see p. 132), can be readily isolated by taking advantage of the fact that it forms a crystalline double salt with copper acetate. They showed that this body also on treatment with hydriodic acid and phosphonium iodide yields hæmopyrrol. This fact establishes, therefore, the intimate chemical relationship between chlorophyll and the blood-pigment, a fact of great biological interest.

Constitution of Hæmopyrrol.—Although pyrrol is an unstable body and difficult to isolate, Nencki and Zaleski succeeded in obtaining a relatively large yield by the reduction of hæmine—in some cases as much as 32 per cent. of the quantity theoretically obtainable. The determination of its constitution, therefore, should throw considerable light on the constitution of hæmatoporphyrin and hæmatine.

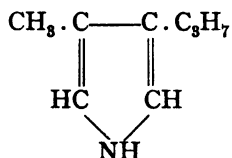
Now it has been already stated that hæmopyrrol gives the ordinary pyrrol reactions; furthermore, Küster has indicated the relationship of the hæmatinic acids prepared by him to pyrrol derivatives; he showed that from the hæmatinic acid $C_8H_9O_4N$, a body $C_7H_9O_2N$ can be prepared to which he ascribed the constitution of a methyl-ethyl-maleic imide.

This leaves for hæmatinic acid the probable formulæ—



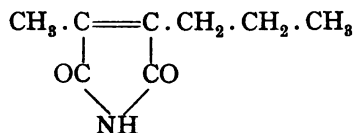
Now hæmatinic acid is obtained from hæmatine by oxidation, whereas hæmopyrrol is obtained by reduction; the latter is either a butyl pyrrol or a methyl-propyl pyrrol. In view of the fact that Küster's hæmatinic acid yields on scission a body containing a methyl group, viz., methyl-ethyl-maleic imide, and in view also of the fact that both hæmatinic acid and hæmopyrrol are obtained in relatively large yields from hæmine, Nencki and

Zaleski were justified in assuming a direct chemical relationship between these bodies, and ascribing to hæmopyrrol a formula containing a methyl group. They considered, therefore, that it was a methyl-propyl derivative, and assigned to it the formula—



i.e., the formula of methyl-propyl pyrrol.

Various attempts have been made recently to confirm this constitution. Küster and Haas (4) have oxidised hæmopyrrol with chromium trioxide in the cold; they expected to obtain from hæmopyrrol a body of the formula—

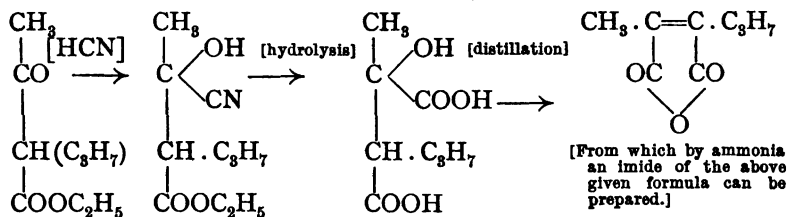


i.e., methyl-propyl-maleic imide. They obtained in very small yield a body melting at 63°-64°.

They also prepared synthetically methyl-propyl-maleic imide by the following method:—

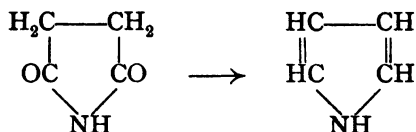
Propyl-ethylacetoacetate was treated with hydrocyanic acid in the cold. The product thus obtained yielded on hydrolysis methyl-propyl-maleic acid. This on distillation yielded methyl-propyl-maleic anhydride, which on treatment with ammonia gives the corresponding imide.

These reactions may be represented by the formulæ—



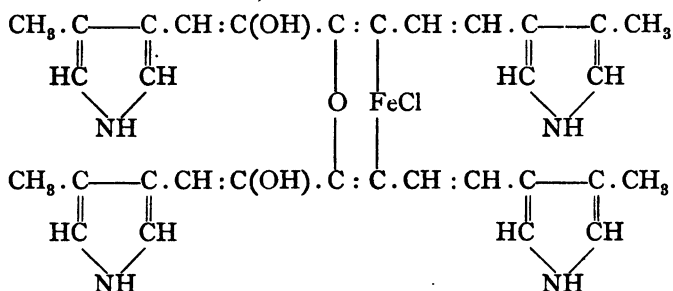
The synthetically obtained imide was found by Küster and Haas to melt at 56° - 57° , *i.e.*, some 7° lower than the product obtained from pyrrol. These researches do not, therefore, establish definitely the constitution of hæmopyrrol. It must be remembered, however, that this body is extremely difficult to prepare in large quantity, and that Küster and Haas obtained its oxidation product only in very small yield. It must be remembered, furthermore, that the imide can exist in stereo-isomeric forms. The fact, therefore, that the synthetic product and the imide obtained from pyrrol have not identical melting-points, is not sufficient evidence for the rejection of the formula proposed by Nencki and Zaleski.

Buraczewski and Marchlewski(1) have attempted to directly synthesise hæmopyrrol. It is known that succinimide on distillation with zinc dust yields pyrrol, if only in small quantities.



It was thought that methyl-propyl-maleic anhydride on similar treatment should yield hæmopyrrol. By this reaction Buraczewski and Marchlewski obtained a product which on oxidation gave very similar colour reactions and absorption spectra to urobilin. The quantity of product obtained was very small, and not free from impurities. On the whole, the bulk of evidence so far obtained seems to strongly favour the original Nencki-Zaleski formula for hæmopyrrol.

Assuming that hæmopyrrol has the constitution of a methyl-propyl-pyrrol, and assuming that one molecule of hæmatoporphyrin is capable of yielding four molecules of hæmopyrrol, it is possible to form some sort of conception of the constitution of hæmine. The one suggested originally by Nencki and Zaleski, on the assumption that hæmopyrrol is methyl-propyl pyrrol, is the following :—

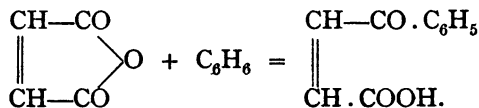


Although this formula is not in accordance with the latest empirical formula assigned to hæmine (which should, according to Küster, contain 34 and not 32 carbon atoms), it probably gives some idea of the true constitutional formula of the chromogenic group of hæmoglobin. When we remember, furthermore, that this large group forms less than 5 per cent. of the hæmoglobin molecule, we can form a conception of the huge size of the proteid molecule. The dehydrochloride of hæmine has the minimum molecular weight of 645. Hæmoglobin cannot, therefore, have a smaller molecular weight than 12,900.

Some Preliminary Experiments on the Lipochromes.

Marchlewski (6) has called attention to the fact that certain lipochromes (fat-pigments) are very similar in their spectra to a series of colouring matters first prepared synthetically by v. Pechmann. These bodies were obtained by the action of anhydrous aluminium chloride on a mixture of a maleic anhydride with a hydrocarbon; a condensation product is thus obtained, from which by the action of water-withdrawing reagents a pigment similar to the lipochromes is prepared.

Maleic anhydride and benzene condense in the presence of aluminium chloride according to the following equation:—



The constitution of the pigments obtained by the action of water-withdrawing agents on these condensation products is not known. Like the lipochromes, they dissolve in concentrated sulphuric acid, yielding a corn-flower blue solution; they give, furthermore, absorption spectra very similar to those of the lipochromes. Now it has long been thought that the lipochromes are analogous in constitution to the blood-pigments, and we know now the relation of the latter to maleic acid derivatives. These facts suggest a method for the synthesis of lipochromes and bodies analogous to the blood-pigments, and Marchlewski proposes to further investigate the Pechmann reaction, and to apply it to the object of obtaining synthetically some of the natural pigments.

We may summarise our knowledge on the pigmentary group contained in hæmoglobin by stating that, whilst the constitution of the group is still unknown, researches have already proceeded so far that we may expect in the not very distant future that problem of the constitution, and the chemical relationship of these interesting and physiologically important bodies will be definitely solved.

REFERENCES—LECTURE VII

- (1) BURACZEWSKI and MARCHLEWSKI.—*H.Z.*, 43, p. 410, 1905.
- (2) KÜSTER.—*H.Z.*, 40, p. 391, 1904.
- (3) KÜSTER.—*Liebig's Annalen*, 315, p. 174, 1901.
- (4) KÜSTER and HAAS.—*Ber.*, 37, p. 2470, 1904.
- (5) LAWROW.—*H.Z.*, 26, p. 343, 1898.
- (6) MARCHLEWSKI.—*H.Z.*, 13, p. 196, 1903.
- (7) NENCKI and MARCHLEWSKI.—*Ber.*, 34, p. 1687, 1901.
- (8) NENCKI and ZALESKI.—*Ber.*, 30, p. 423, 1890.
- (8a) NENCKI and ZALESKI.—*Ber.*, 34, p. 997, 1901.
- (9) SCHUIZ.—*H.Z.*, 24, p. 443, 1897.
- (10) SCHUNCK and MARCHLEWSKI.—*Liebig's Annalen*, 278, p. 329, 1894; 284, p. 81, 1895; 288, p. 209, 1895; 290, p. 306, 1896.
- (11) ZALESKI.—*H.Z.*, 37, p. 54, 1902.

LECTURE VIII

THE INTERMEDIARY DEGRADATION PRODUCTS OF THE ALBUMENS ; THE SYNTHESIS OF THE POLYPEPTIDES

THE degradation products of the albumens which we have been considering in the previous lectures are all bodies of a relatively small molecular weight and comparatively simple chemical nature. It is impossible, however, to form a conception of the constitution of an albumen merely by the isolation of a large number of the ultimate degradation products obtainable from it by hydrolysis with acids ; we must know also the method by which these products are condensed together in the larger molecule, and for this purpose it is necessary that we should be able to isolate more complex degradation products, which can in their turn be degraded into the simpler bodies. Now if we can isolate several such complex degradation products, of which we can determine the constitution, we shall be in a better position to discuss the actual constitution of an albumen.

The problem as it now presents itself to us is analogous to that presented to chemists in the seventh and eighth decades of the last century with regard to the constitution of uric acid ; we shall probably arrive at a solution of our problem by proceeding on similar lines to the investigators, to whose labours the final knowledge of the constitution of uric acid is due.

It may be recalled here, that the first degradation products of uric acid synthesised were simple mono-ureides like parabanic acid ; the knowledge thereby obtained led shortly afterwards to the synthesis of the more complex degradation products, which

in their turn yield the simpler products on degradation; the knowledge acquired from the determination of the constitution of the more complex degradation products led finally to the determination of the constitution of uric acid itself.

It has already been mentioned that several chemical methods are applicable to the hydrolysis of albumens. By the use of strong mineral acids the ultimate products of hydrolysis are obtained. If we employ, however, gentler reagents, such as the enzymes acting at the temperature of 37° , we can obtain bodies of much greater complexity than the simple amino- and diamino-acids; we can obtain products which still possess many of the characteristics of an albumen, and which themselves, on hydrolysis with stronger reagents, or by prolonged action of enzymes, yield the ultimate products of hydrolytic degradation.

These complex intermediary products are known as the albumoses and peptones; as an albumen on digestion with enzymes yields a mixture of bodies of this class, it is important that we should have methods available for their separation.

Separation of the Albumoses and Peptones.

Kühne and his pupils, especially Chittenden and Neumeister, were the first to devise systematic methods for the separation of the albumoses and peptones; they took advantage of the fact that various members of this class can be precipitated by salts, and that the more complex bodies can be more readily precipitated than the simpler bodies. This method of separation of substances of albumen nature has been already discussed.

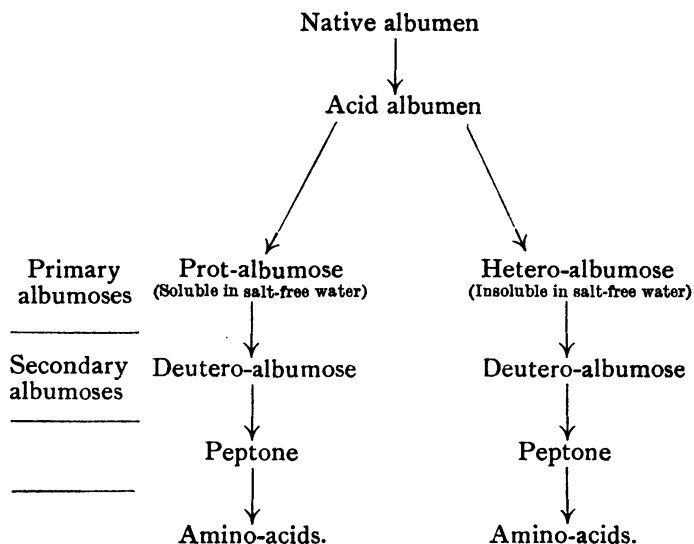
According to Kühne, the intermediary degradation products of an albumen produced by peptic digestion can be represented by the scheme shown on page 141.

The acid albumen is precipitated on neutralisation of the solution (previously acidified with hydrochloric acid for peptic digestion).

The primary albumoses are precipitable by saturation of their solutions with magnesium sulphate or sodium chloride.

Secondary albumoses are precipitable by saturation with ammonium sulphate. Peptones cannot be precipitated by salts.*

As far as the main points are concerned, the scheme of Kühne has been generally accepted.



Recent work of Hofmeister's pupils.—Various modifications of the methods of separation have been introduced in recent years by Hofmeister and his pupils; reference has already been made to the work of Pick and Zunz.

The chief modification consists in the employment of one, and only one salt as a precipitant, either ammonium sulphate (Pick) or zinc sulphate (Zunz).

The scheme elaborated by Pick for the separation of the products of peptic digestion of fibrin (Witte's peptone) is given in the table on p. 13.

In order to determine the limits of precipitability, Pick took

* See classification of precipitating salts, p. 11.

2 c.c. of a solution of a peptone and added to it 8 c.c. of a mixture of varying quantities of distilled water and saturated ammonium-sulphate solution. If to 2 c.c. of peptone solution be added a mixture, *e.g.*, of 7 c.c. distilled water and 1 c.c. saturated ammonium sulphate, a solution containing approximately 10 per cent. of salt necessary for complete saturation would be obtained; if to 2 c.c. peptone solution a mixture of 5 c.c. water and 3 c.c. saturated ammonium sulphate be added, a solution containing 30 per cent. of salt necessary for complete saturation would be obtained.

In a series of preliminary experiments, Pick determined the concentrations of ammonium sulphate necessary to produce precipitation. For this purpose tests were made with 8 c.c. of liquid containing varying proportions of distilled water and ammonium-sulphate solution, which were added to portions of 2 c.c. of peptone solution; after standing for some time, the mixtures were filtered in event of a precipitate being formed, and a drop of ammonium sulphate was then added to the filtrate to determine whether precipitation was complete.

In the experiments carried out with Witte's peptone, Pick found that precipitation commenced when ammonium sulphate was added in such quantities that the solution contained 24 per cent. of the salt necessary for complete saturation. (In test experiment, 2 c.c. peptone + mixture of 2.4 c.c. saturated ammonium-sulphate solution and 7.6 c.c. water.)

On addition of more ammonium sulphate to the solution, precipitation continues until 42 per cent. of the salt necessary for complete saturation has been added (2 c.c. peptone, 4.2 c.c. saturated ammonium sulphate, 3.8 c.c. water). The filtrate from the precipitate, after the addition of this amount of salt, does not give any further precipitate when a small amount of ammonium sulphate is added; if, however, ammonium sulphate be added until the liquid is 54 per cent. saturated, precipitation recommences, to cease when saturation reaches the 62 per cent. limit; a third precipitate can be obtained when 70

per cent. of the salt necessary for complete saturation has been added.

Altogether, four distinct fractions were obtained by Pick by precipitation with ammonium sulphate, viz. :—

(i) The hetero- and prot-albumose fraction, which is completely precipitated when 42 per cent. of the ammonium sulphate necessary for complete saturation has been added.

(ii) From the filtrate from the above an A-albumose fraction can be obtained. This is completely precipitated at 62 per cent. saturation.

(iii) From filtrate of (ii) a B-albumose fraction is obtained, and is completely precipitated at 95 per cent. saturation.

(iv) From filtrate (iii) a C-albumose is precipitated on complete saturation with addition of acid.

Pick has found that this method of fractional precipitation is more satisfactory than the older method of Kühne and his school, for he shows that the precipitation by sodium chloride of the prot-albumoses is not by any means complete.*

Kühne separated his primary albumoses into two further fractions by dialysis, viz., a prot-albumose fraction which remains in solution, and a hetero-albumose fraction which separates out. Pick discards this method, and separates the first fraction produced by ammonium-sulphate precipitation into new fractions by taking advantage of the fact that there is a great difference in the solubilities of the products of the precipitation in alcohol; a hetero-albumose fraction was obtained which was insoluble in 32 per cent. alcohol, and a prot-albumose fraction which was soluble in 80 per cent. alcohol.

In a similar way the fractions containing the A, B, and C albumoses were separated into smaller fractions by means of alcohol of varying strength; the details of these separations have been already given on p. 13.

The fractions obtained by the two combined methods of separation (*i.e.*, fractional precipitation by ammonium sulphate, and fractional solution with graded strengths of alcohol) show

* It is also very unlikely that the separation by Pick's method is complete.

markedly different chemical reactions, and different percentage chemical compositions. It has already been noticed that one of the B-albumose fractions gives a very marked reaction when treated with the Molisch-Udransky reagent, and contains, therefore, a large amount of carbohydrate groups; this fraction has been called by Pick the glyc-albumose. Another fraction containing relatively large amounts of sulphur is known as the thio-albumose.

Differences in qualitative reactions such as these (which are given in detail in the table on p. 13) are *prima facie* evidence of the fact that a separation of products has taken place; they demonstrate the possibility of degrading the albumen molecule into groups which are still very complex, and which differ chemically from one another; if, now, we can isolate such groups and follow out by a similar method of hydrolysis and separation their degradation into secondary products, and the degradation of the secondary products into tertiary products, and these latter into simpler products, and so on until we arrive at the amino-acids, the final degradation products, a method is indicated for the determination of the constitution of an albumen.

Pick himself has already submitted some of the fractions obtained by him to further investigation; he has examined the final degradation products obtained by acid hydrolysis from hetero- and prot-albumose, and found the following differences:—

<i>Hetero-albumose</i>	<i>Prot-albumose</i>
contains 39 per cent. of the total nitrogen in basic form.	contains 25 per cent. total nitrogen in basic form.
yields small amount of tyrosine, or bodies capable of yielding skatol or indol.	yields large amount of tyrosine, and bodies yielding skatol and indol.
„ large quantities of leucine.	„ very little leucine.
	„ no glycocoll.

These results have been obtained by using only very small quantities of the fractions. Before researches of this nature are complete, it will be necessary to submit each fraction to acid hydrolysis, and estimate the products by the method of E. Fischer in as nearly a quantitative manner as possible.

Pick's results have been obtained from Witte's peptone, which is a commercial product, and which cannot be expected to be always homogeneous; furthermore, it is impossible to determine with a product such as this whether the products which can be separated by fractionation are of primary, secondary, or tertiary, etc., nature. For this purpose, it is necessary to follow out quantitatively the course of peptic digestion of fibrin. This has been done by Pick, who has found that hetero-albumose and prot-albumose and albumose B are found in the earliest stages of digestion. He concludes, therefore, that all these products are of primary nature, and that albumose B is not apparently formed from hetero- or prot-albumose, but directly from the fibrin itself. This is a fact of great interest, in that this particular albumose is very rich in carbohydrates.

Pick has also examined the products of enzyme hydrolysis of hetero- and prot-albumose with the object of isolating their intermediary degradation products; this research has, however, been only carried out on a small scale.

As, in the determination of the constitution of an albumen, it is of such obvious importance to obtain the intermediary degradation products in order, a further elaborate research on the course of peptic digestion has been undertaken by another pupil of Hofmeister, viz, Zunz.

Zunz has employed, instead of ammonium sulphate, zinc sulphate in acid solution; a reagent originally suggested by Baumann and Bömer. This reagent is made by saturating with zinc sulphate a mixture of 100 parts water with 2 parts dilute sulphuric acid (1 volume concentrated acid, 4 volumes water). The method of experiment was similar to that adopted by Pick with ammonium sulphate, and similar fractions were obtained. The following table shows the analogy between

K

the results obtained from Witte's peptone (5 per cent.) with ammonium sulphate and zinc sulphate (acid):—

	Fraction I.		Fraction II.		Fraction III.	
	Am ² SO ⁴ .	ZnSO ⁴ .	Am ² SO ⁴ .	ZnSO ⁴ .	Am ² SO ⁴ .	Zn ² SO ⁴ .
Lower limit .	26	24	54	52	72	62
Higher limit of precipitation .	44	46	62	60	95	72

These numbers represent the percentage of complete saturation.

A fourth fraction is in each case obtained on complete saturation. When zinc sulphate is used as a precipitant, better results are obtained by working with more dilute solutions, as in these cases there is a greater interval between the fractions. If, *e.g.*, a 2 per cent. peptone solution be employed, the fractions are obtained between the following limits :—

Fraction I. Between 30 and 46 per cent. of complete saturation.

" II. " 58 and 64 per cent. " "

" III. " 72 and 82 per cent. " "

" IV. " 86 and 100 per cent. " "

By this use of zinc sulphate in acid solution, Zunz has obtained results analogous to those obtained by Pick with ammonium sulphate. He has employed this fractionation method for following out the course of peptic digestion of several albumens, such as crystallised egg and serum albumens, casein, eu- and pseudo-globulin.

These products were all coagulated with hot alcohol, and then suspended in 0.3 per cent. hydrochloric-acid solution, containing 0.4 gram Grüber's pepsine per litre. At stated intervals portions of the digestive liquid were taken out and fractionally precipitated by acid zinc sulphate. The nitrogen was estimated in an aliquot part of the liquid of the filtrate after each precipitation, the nitrogen in the precipitate being thereby determined by difference. The following fractions were obtained :—

I. An acid albumin fraction, which is fully precipitated before the 20 per cent. limit of saturation is reached.

II. The proto- and hetero-albumose fraction, produced by half saturation.

III. Deutero-albumose A fraction, produced by $\frac{2}{3}$ saturation with crystallised serum albumen and caseine, $\frac{1}{10}$ saturation with crystallised egg-albumen, pseudo-globulin and eu-globulin, and 73 per cent. saturation with serum globulin.

IV. Deutero-albumose B, produced by $\frac{4}{7}$ saturation with serum albumen and caseine, 83 per cent. saturation with egg-albumen, 85 per cent. saturation with serum globulin, pseudo- and eu-globulin.

V. Deutero-albumose C. Full saturation with powdered zinc sulphate.

VI. Peptones and basic products precipitated by phosphotungstic acid.

VII. Nitrogenous residue.

The adjoining table (p. 148) shows the course of digestion of crystallised serum albumen, in two series of experiments, and will serve as an example of the results obtained by Zunz.

The first series of experiments shows the progress of digestion during the first two hours. The numbers in thick type indicate the maxima in the second series of experiments, which show the course of digestion in intervals between four hours and thirty days. Similar results were obtained with other albumens.

It will be observed that Zunz finds as primary products of peptic digestion acid albumen, which disappears within the first four hours of enzyme action, hetero- and prot-albumose, and also albumose B. In this respect he fully confirms the results of Pick. Hence, we must discard the original scheme of Kühne, which places all the deutero-albumoses amongst the secondary products of digestion.

The occurrence of this B-albumose fraction amongst the primary products is a very constant phenomenon, which has been observed in the case of all the albumens in which the course of peptic digestion has been investigated. In the case

* PERCENTAGE OF NITROGEN CONTAINED IN

Time of Digestion.	Acid Albumen.	Albumoses.					Other Products, including Peptones.			Bluret Reaction of Filtrate from Albumoses.
		Primary Albumoses.	A.	B.	C.	Total Albumose.	Products precipitable by Phosphotungstic Acid.	Products not precipitable by Phosphotungstic Acid.	Total.	
			Deutero-albumose.	Deutero-albumose.	Deutero-albumose.					
1/2 hour	-	44.03	0.00	39.15	0.00	83.18	2.64	9.60	12.24	Negative.
1 hour	-	42.65	1.75	34.00	2.10	80.50	1.56	12.09	13.65	Positive.
2 hours	-	39.89	3.02	35.20	3.14	81.25	2.89	13.08	15.97	Positive.
4 hours	-	87.10	4.93	29.48	3.28	74.77	1.83	23.40	25.23	...
8 hours	-	30.12	8.64	25.82	4.86	69.44	2.65	27.91	30.56	...
22 hours	-	14.94	7.12	16.51	5.80	44.37	5.12	50.51	55.63	...
2 x 24 hours	-	7.86	5.26	28.49	6.98	43.59	7.89	48.52	56.41	...
3 x 24 hours	-	3.71	3.05	18.95	8.28	33.99	19.06	46.95	66.01	...
6 x 24 hours	-	1.58	1.17	6.84	7.95	17.54	39.08	43.38	82.46	...
10 x 24 hours	-	0.64	0.00	2.96	6.64	10.24	...	...	89.76	...
15 x 24 hours	-	0.00	0.00	0.89	6.28	7.17	57.67	35.16	93.83	...
21 x 24 hours	-	0.00	0.00	0.00	6.17	6.17	55.64	38.29	93.83	...
30 x 24 hours	-	0.00	0.00	0.00	5.89	5.89	63.09	31.02	94.11	...

* It has been recently shown that simple amino-acids are partially carried down by the various albumose and peptone precipitates. The numbers given in the above are probably, therefore, not strictly accurate.

of fibrin it contains, as Pick has shown, a considerable part, if not all, of the carbohydrate groups of the albumen molecule. As it is probable that this fraction is not always a glyc-albumose. Hofmeister has suggested for it the general name of "syn-albumose."

The other deutero-albumoses (A and C) appear to be secondary products. It would appear also that part of albumose B is of secondary nature, for it is found in its maximum yield at a very early stage of digestion, and decreases rapidly in quantity (in the case of serum albumen) during the first twenty-two hours of digestion; after this interval it again increases in quantity, reaching a second maximum after two days.

Another point of interest is the fact that products which cannot be precipitated either by salts or by phosphotungstic acid are produced in the very earliest stages of digestion. It is possible, therefore, that amino-acids are also primary products of digestion (see table, p. 148).

Although so much labour has been expended on the above researches, the investigations can only be considered to be still in their infancy. The course of peptic digestion of all the intermediary products remains to be determined in detail; it will be also necessary to determine the ultimate products of hydrolysis by acids of each fraction, and to isolate and identify the simpler degradation products obtained in the different stages of peptic digestion, of which the nitrogen numbers are given in three columns in the table on p. 148.

More especially important is it that the simpler products obtainable in the earliest stages of digestion of any body (whether native albumen, primary or secondary product) should be identified, and that the order in which the various amino-acids are eliminated from the molecule should be determined, for it is obvious that the data derivable from an investigation of this kind will throw considerable light on the way in which the various amino-acids, etc., are coupled together in the albumen molecule.

The Isolation of the Simplest Intermediary Products of Proteid Degradation.

Whilst many investigators have been occupied with the isolation of the most complex degradation products, such as the albumoses, by the methods already described, others have attempted the isolation of the simplest intermediary products, bodies, *e.g.*, which are not precipitable by salts and which on acid hydrolysis yield only two or perhaps three final degradation products. Both classes of research may be regarded as different stages in one investigation; one set of workers has investigated the degradation products not far removed from the original albumen, the other set, the degradation products not far removed from the ultimate products of hydrolysis. The former attempt to pass from the more complex to the simple, the latter from the more simple to the complex.

The number of known simple intermediary bodies which cannot be precipitated from solution by salts, and which give the biuret reactions (peptones proper), is very small. Salaskin claims to have obtained leucinimide by the peptic and tryptic digestion of hæmoglobin.

Langstein, by the peptic digestion of blood-serum, isolated an acid of which the calcium salt had the formula $C_9H_{16}N_2O_9Ca_2$. This was obtained in very small quantity, and appeared to yield on hydrolysis with acid a basic body and a mono-amino-acid.

Siegfried has also carried out several investigations with the object of isolating peptones.

Siegfried's Researches.—Kühne and his pupils isolated from amongst the bodies obtained by the action of trypsin on egg-albumen a product which could not be precipitated by salts, but which still gave the biuret reaction. A similar product was obtained by Balke from fibrin. These bodies, owing to their supposed resistance to the tryptic enzyme, have been called anti-peptones. Siegfried has reinvestigated these bodies, and introduced new methods for their preparation, by means of which products of constant chemical composition have been obtained.

The products are isolated in the first instance in the form of their iron salts, by means of a fractional precipitation with iron alum.

The method of preparation of anti-peptones according to Siegfried is as follows:—The albumen body is suspended in ten to twenty times its weight (calculated as dried substance) of 0.3 per cent. sodium carbonate solution. Strong trypsin solution is then added, and the mixture is then digested for some weeks at 37°. As soon as the saturation of a test portion of the liquid with saturated ammonium sulphate gives only a slight precipitate of albumose, the digestion is stopped. The liquid is neutralised with sulphuric acid, and filtered after standing for one day. It is then saturated with ammonium sulphate at room-temperature, and then, after standing for one day longer, again filtered. To this filtrate a mixture of three parts of concentrated ammonium sulphate and one part of concentrated sulphuric acid are added, until a further addition produces neither turbidity nor precipitate. The addition of acid ammonium sulphate is necessary to complete the precipitation of the albumoses. Ammonium sulphate solution previously saturated with gaseous ammonia is then added to the filtrate from the last albumose precipitate till the reaction is only faintly acid. A saturated solution of ferric ammonium alum in ammonium sulphate is then added, until precipitation is complete. By this means a copious precipitate is produced which is the iron salt of one anti-peptone; it is called by Siegfried iron precipitate No. I. To the filtrate from this iron salt a saturated solution of iron alum in ammonium sulphate equal in bulk to about one-tenth of that necessary to produce precipitate I. is added, and the mixture is then just neutralised with concentrated ammonia; a precipitate is produced which is a mixture of the iron salt of two anti-peptones. To the filtrate from this intermediary precipitate *powdered* iron alum is added, with continuous stirring, and then concentrated aqueous ammonia, the latter only in such quantity as to produce a faintly acid solution. The liquid should never be quite neutralised. In this way a second

precipitate, called by Siegfried iron precipitate No. II., is produced.

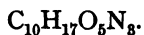
The two iron precipitates I. and II. are subjected to further purification; precipitate I., after thoroughly washing, is suspended in concentrated ammonium sulphate; ammonia is added to the mixture, and the whole is digested at 40°; the precipitate formed is washed with water; washings and filtrates are saturated with ammonium sulphate, and from this solution the iron compound is reprecipitated by the method already described, after separation of the last traces of adhering albumoses with acid ammonium sulphate. It is then decomposed by ammonia and baryta, the latter being added only in slight excess; to the filtrate from the iron hydrate and barium sulphate ammonium carbonate is added to get rid of the last traces of barium; the filtrate is then evaporated to a syrup at 40°. This syrup contains ammonium salts; it is dissolved in 12 per cent. acetic acid, and alcohol is added to the solution until a faint permanent turbidity is produced. The liquid is then poured into a large quantity of absolute alcohol (1 litre to 20 c.c. of the solution), and a snow-white precipitate is thereby produced. That obtained from iron precipitate I. is called by Siegfried anti-peptone β .

A similar, though not quite identical procedure, is adopted for preparing the so-called α -anti-peptone from iron precipitate No. II. To anti-peptone β , prepared from fibrin, Siegfried assigned the formula



It is a monobasic acid, a fact which was determined by the analysis of several of its salts.

The anti-peptone α , also prepared from fibrin (or Witte's peptone), Siegfried assigned the formula



Both bodies are lævo-rotatory, and the rotation is a criterion of the purity of a preparation. They give a marked biuret reaction. These bodies on acid hydrolysis yielded each a basic body and amino-acid. The former is apparently lysine (lysa-

tanine ?), in the case of both peptones ; the α -peptone yields in addition aspartic acid, the β -peptone glutamic acid.

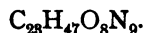
A similar peptone obtained from gelatine yielded on hydrolysis lysine, arginine, glutaminic acid, and glycocoll.

Kyrines.—In addition to the acid peptones, Siegfried has also recently isolated peptones of basic character, which he calls *Kyrines*.

These bodies can be prepared by the hydrolysis of albumens with about 12 per cent. (sometimes 16 per cent.) hydrochloric acid at 38° - 39° .

In the case of caseine, the digestion is continued for three weeks ; the albumen is mixed with ten times its weight of 12 per cent. acid, and the whole is repeatedly shaken from time to time ; to the filtered liquid after this period of digestion, phosphotungstic-acid solution is added, at first in 10 per cent. solution, and afterwards 50 per cent. The precipitate thus produced, after filtering off from the mother-liquor and washing with 5 per cent. sulphuric acid, is dissolved in ammonia, at 30° - 40° ; baryta is added, the barium phosphotungstate produced is filtered off, and ammonium carbonate is added to the filtrate ; after filtration again, and evaporation of the filtrate at 40° , a syrup is obtained, which consists principally of the crude kyrine. This can be decolourised by the addition of lead-acetate solution to a solution of the syrup, filtration from the precipitate, and elimination of the excess of lead by means of sulphuretted hydrogen. The filtrate from the lead sulphide is concentrated to a syrup. This is dissolved in 5 per cent. sulphuric acid, and the acid solution is then thrown into a large amount of alcohol (1 litre of alcohol to each 15 c.c. of solution). The kyrine sulphate is thereby precipitated. The product thus obtained is generally dissolved and reprecipitated several times, to completely purify it.

Caseokyrine appears to have the following formula :—



Its sulphate has the formula



The free base is amorphous ; it yields, however, a crystalline phosphotungstate ; it is optically inactive ; it gives the biuret reaction, but not so strongly as the antipeptones.

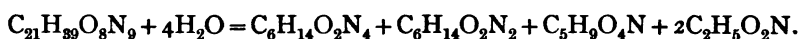
On acid hydrolysis it yields basic bodies together with glutaminic acid ; the former were determined quantitatively by the method of Kossel and Kutscher ; they consisted of arginine and lysine, in the proportions of one molecule of the former to two of the latter. The hydrolysis of caseokyrine may be represented, therefore, by the following equation :—



A similar body has also been prepared by Siegfried from gelatine. To this body, the so-called glutokyrine, the following formula has been assigned :—



On hydrolysis with acids, it yields one molecule of lysine, one molecule of glutaminic acid, and probably two of glycocoll. This hydrolysis may be represented, therefore, by the following equation :—



This equation has not, however, been determined with absolute certainty.

Fischer's Experiments on the Isolation of Intermediary Degradation Products.

It was observed in E. Fischer's laboratory that caseine on acid hydrolysis yielded appreciable quantities of pyrrolidine-carboxylic acid ; if the caseine be treated, however, even for several months, with pancreatic enzymes under the ordinary conditions, it yielded hardly a trace of the pyrrolidine acid. The digestive liquid, on the other hand, when hydrolysed with mineral acids, gave the pyrrolidine-carboxylic acid in almost the same yield in which it could be obtained on subjecting the caseine directly to acid hydrolysis. Fischer has concluded, therefore, that the pyrrolidine-carboxylic acid is found combined with

other amino-acids in the form of a peptone-like body, which is very resistant to enzyme hydrolysis.

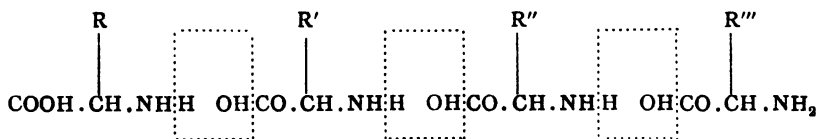
The same results were obtained when edestine, hæmoglobin, egg-albumen, fibrin, and serum globulin were submitted to tryptic digestion—a peptone-like residue was always obtained which was resistant to enzyme hydrolysis, and which on acid hydrolysis yielded fairly large quantities of pyrrolidine-carboxylic acid. The proline-yielding* body could be precipitated in the form of a phosphotungstate; the product obtained from the latter in the usual way yielded on hydrolysis, besides proline (in the case of caseine), alanine, leucine, glutamic and aspartic acids, and phenylalanine.

This proline-yielding complex has not yet been obtained pure.

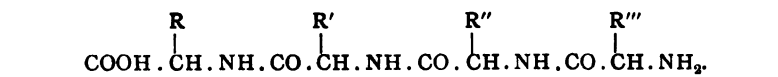
Researches of E. Fischer on the Synthesis of the Peptides and Polypeptides.

So far, it will be seen that few simple intermediary products of albumen degradation have been directly isolated. E. Fischer and his pupils have attempted to throw light on the nature of such bodies by synthesising various condensation products of amino-acids, and comparing the properties of the synthetic bodies with the products obtained directly by albumen degradation, especially as regards their colour reactions and their capacity for hydrolysis by enzymes.

Hofmeister had already suggested that amino-acids were conjugated together in the albumen molecule by the elimination of water between the carboxyl group and the amino-group, thus—



to give a body of the general formula—



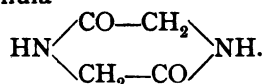
* Pyrrolidine-carboxylic acid.

Such bodies have been designated by E. Fischer polypeptides. If formed by the condensation of two amino-acid groups they would be called dipeptides, if from three groups tripeptides, etc. Great interest attaches, therefore, to this class of bodies, from the fact that all chemical evidence indicates that they are formed by the degradation of the albumen molecule; their synthesis, therefore, is the first stage in the artificial construction of an albumen molecule.

The method of synthesis has been gradually evolved in a series of researches by E. Fischer and his pupils.

Before E. Fischer, the condensation of amino-acids had been observed by many investigators; the polyaspartic acids, for example, had been prepared and described by Schiff, and the conversion of glycocoll into a horny amorphous body by heating with glycerine had been investigated by Balbiano and Frasciatti.

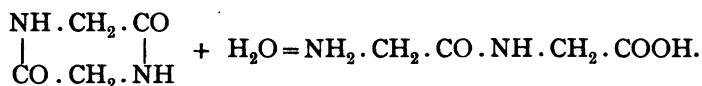
Of the simple condensation products, the earliest described was leucinimide, which was prepared by Bopp in 1849 directly from leucine, by heating the latter in a stream of carbonic acid or hydrochloric acid gas. The anhydride of glycocoll was prepared by Curtius, who determined its molecular weight, and assigned to it the formula



Other anhydrides of a similar class have also been prepared. They are known generally as α - γ - or 2-5-diacipiperazines. These diacipiperazines form the starting-point for the researches of E. Fischer and his pupils. These bodies were prepared by E. Fischer from the esters of the amino-acids (prepared by the method given in Lecture III., p. 43), by heating them to 170° in a sealed tube. This heating is not necessary in the case of glycocoll, for Curtius has shown that glycocoll ester is converted into the piperazine body simply on standing at ordinary temperatures.

Fischer and Fournau have shown that if a piperazine be heated for a few minutes with concentrated hydrochloric acid, scission of the ring takes place, and the hydrochloride of an

amino-acid is obtained. In the case of the piperazine obtained from the ester of glycocoll, the reaction may be represented as follows :—*



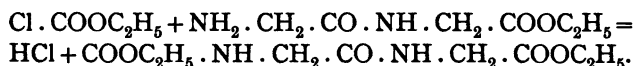
Similar reactions take place with the more complex bodies such as leucine and alanine.

For the radical $\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CO}$. Fischer proposes the name "glycyl." The body obtained by the action of hydrochloric acid on diacipiperazine, according to the above equation, would be called in Fischer's nomenclature "glycylglycine."

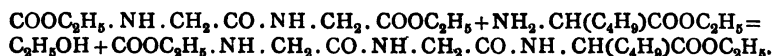
The free acid, as well as its ester, can be readily reconverted into the anhydride from which it is formed; under the new nomenclature, the latter should be called "glycine anhydride."

First Method of Synthesis.—Glycylglycine is a peptide of the simplest form, produced by the condensation of two molecules of one amino-acid. The first attempt to obtain a more complex body was made by heating together the ester of one amino-acid with the ester of another amino-acid. In one of the amino-acid esters an amino-group was protected from condensation by first treating the body with ethyl chlorcarbonate. In this way a carboxethyl-amino-derivative was obtained, and one amino-group was thereby protected from condensation.

Example.—Glycylglycine is treated with chlorcarbonic ester; carboxethylglycylglycine is thereby produced—



If, now, this ester be heated for thirty-six hours with the ester of leucine, carboxethylglycylglycine-leucine ester is produced—



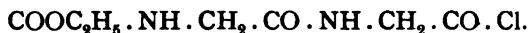
* A simpler method has since been discovered. The anhydride, on shaking with an equimolecular quantity of normal caustic soda, gives the sodium salt of the peptide. These sodium salts can be directly condensed with acid chloride for the purpose of synthesis of Method III., *Ber.*, 38, 607.

We have now a derivative of a polypeptide produced by the condensation of the three amino-acid groups.

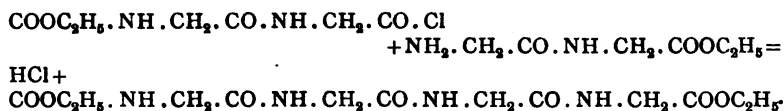
The yields obtained by this method of synthesis were poor.

Second Method of Synthesis.—The second method of synthesis consisted in the treatment of the esters of the carboxethyl-amino-derivatives with thionyl chloride (SO_2Cl_2). An acid chloride is thereby produced, which readily condenses with amino-acid esters to form a polypeptide derivative of greater complexity.

Carboxethylglycylglycine, on treatment with thionyl chloride, gives the body



This condenses with glycylglycine ester according to the following equation :—



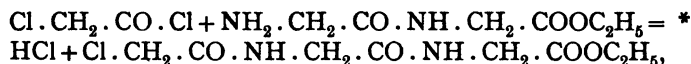
In this reaction we have no less than four glycyl groups condensed together. This constitutes, then, the synthesis of a tetrapeptide derivative.

Third Method of Synthesis.—In both the above methods of synthesis, carboxethyl derivatives of the polypeptides are obtained. There is no method known of eliminating this carboxethyl group, so that neither of the above syntheses lead to a direct synthesis of a polypeptide.

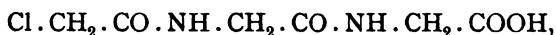
To eliminate this difficulty another synthetical method was proposed. A body of the glycine ester, or glycylglycine ester type, is treated with another body of the chloracetyl chloride type. A chlor-ester is thereby obtained by condensation; this body on very careful hydrolysis under certain conditions * yields the corresponding acid, which on treatment with concentrated ammonia yields an amino-derivative by the replacement of the chloride by the amino-group; this amino-derivative is a true polypeptide.

* *Vide Ber.*, 36, p. 2106, 1903.

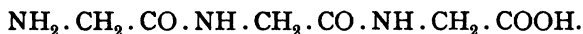
Example.—Chloracetyl chloride reacts with glycylglycine ester thus :—



which on hydrolysis yields



which on treatment with ammonia yields

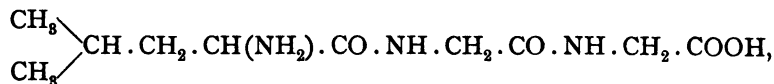


i.e., diglycylglycine ester, a true poly(tri)peptide. In a similar way other bodies than chloracetyl chloride can be employed, such as brompropionyl bromide.

As other examples, we may quote the synthesis of alanyl-glycyl-glycine,



from brompropionyl bromide and glycylglycine ester, leucyl-glycylglycine,



from α -bromcapronyl bromide and glycylglycine ester.

This method of synthesis can be applied to the synthesis of the higher peptides; in fact, Fischer states that the products of reaction are more easy to isolate in many of the cases of the more complex condensation products.

The following are examples of some of the polypeptides produced by this method :—

Dipeptides.

Glycylalanine.

Leucyl-leucine (from leucine and α -bromisocapronyl bromide).

Glycyl *L*-tyrosine.

Leucyl *L*-tyrosine.

* This condensation has in some cases been carried out directly with the sodium salts of the acids instead of the esters.

Tripeptides.

Diglycylglycine (from glycylglycine and chloracetyl chloride).
 Phenylalanylglycylglycine (from α -bromophenylpropionyl chloride and glycylglycine).
 Leucylglycylglycine.

Tetrapeptides.

Triglycylglycine (from diglycylglycine and chloracetyl chloride).
 Dileucylglycylglycine (from bromcapronyl chloride and leucylglycylglycine).

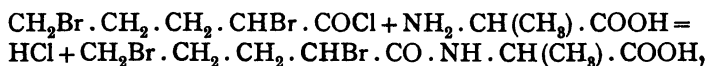
Pentapeptides.

Tetraglycylglycine (from chloracetyl chloride and triglycylglycine).

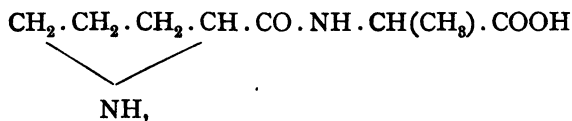
It is of interest to note that optically active forms can be synthesised by the use of amino-acids, which are themselves optically active. (*Cf.* various tyrosine derivatives given above.)

A case of special interest in the application of the above synthesis is the preparation of polypeptides containing the proline group. If the α - δ -dibromvaleryl chloride be condensed with an amino-acid, a dibrom-condensation product is formed. This dibrom-derivative on treatment with ammonia gives a proline derivative.

Example.— α - δ -dibromvaleryl chloride condenses with alanine according to the following equation:—



which on treatment with ammonia gives directly



i.e., prolylalanine.

*Fourth Method of Synthesis.**—Polypeptides in which a

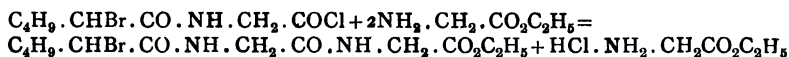
* Published in the interval between the delivery and preparation of lectures for press.

hydrogen atom of an amino-group is substituted by an acetyl radical in which a hydrogen is replaced by a halogen,* yields on treatment with phosphorus pentachloride a substituted acid chloride of the peptide. This can be made to condense with other bodies containing the amino-groups.

Example.—Bromcaprylglycine (an intermediate product of synthesis in the preparation of leucylglycine) gives with phosphorus pentachloride the body



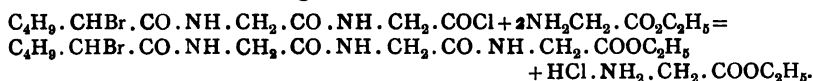
This condenses with glycine ester according to the following equation:—



From this body, by hydrolysis and by subsequent treatment with ammonia, leucylglycylglycine can be prepared.

This reaction is also applicable to more complex bodies.

Furthermore, α -bromcaprylglycylglycine can itself be treated with phosphorus pentachloride to give the corresponding acid chloride, and this again condensed with an amino-acid, thus—



In this way a tetrapeptide can be produced by hydrolysis and subsequent treatment with ammonia. In this way a method is indicated for building up a polypeptide from the carboxyl end of the molecule from an intermediary stage in the synthesis according to Method III.

Fifth Method of Synthesis.†—Amino-acids themselves yield, it has been quite recently shown, acid chlorides on treatment with a mixture of acetyl chloride and phosphorus pentachloride. In this way bodies like leucyl chloride (in the form of its hydrochloride),



can be produced. These can be directly condensed with another

* Intermediate products in synthesis according to Method III.

† Also published since the delivery of these lectures.

amino-acid to form polypeptides. This method is by far the simplest and most direct for the preparation of the polypeptides, as its discovery is of very recent origin, and it has so far been applied to only a very limited number of cases.

These methods of synthesis have been applied not only to simple amino-monobasic acids, but also to amino-dibasic acids such as glutamic and aspartic acids, and polypeptides derived from these bodies have also been prepared; various derivatives from cystine have also been synthesised.

As the synthetic products contain asymmetric carbon atoms, the syntheses have an additional interest from the point of view of stereochemistry. Experiments are being now carried out by Fischer and his school, with the object of preparing optically active bodies in this group.

The General Properties of the Polypeptides: their Hydrolysis by Means of Enzymes.

The synthetically prepared peptides are analogous in their properties to the natural products obtained by the hydrolysis of the albumens; they are bodies which are easily soluble in water, and yield the biuret reaction; they are also in certain cases hydrolysed on digestion with enzymes.

Their reactions to the pancreas enzymes have formed the subject of a research by Fischer and Bergell, of which a brief summary is given below.

It has been found that all polypeptides do not react in the same way on treatment with enzymes; some peptides containing a tyrosine group are readily hydrolysed on treatment with pancreatin, with elimination of tyrosine; other bodies containing glycocholic groups, such as glycylglycine, are extremely resistant; in other cases, when the peptide is a racemic form, the action proceeds asymmetrically, and only one-half of the racemic form is attacked, as, *e.g.*, in the case of carboxyethylglycyl *d*-*L*-leucine. When this body is submitted to the action of trypsin, a scission takes place of the *lævo*-rotatory leucine only, and carboxyethylglycyl *d*-leucine is obtained as a by-product. The action of

trypsin on a peptide depends therefore on several factors, of which the following are the chief:—

(a) The nature of the amino-acid. Certain groups such as tyrosine are especially susceptible to the action of the enzyme.

(b) The stereochemical configuration of the molecule.

(c) The general constitution of the whole molecule.

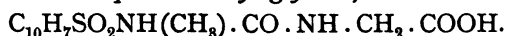
The action of trypsin has been investigated not only on the peptides themselves, but, for the sake of simplicity in the manipulation, also on certain derivatives, such as carboxyethyl, and β -naphthalene sulpho-derivatives.* The action of pancreatin on the following bodies has been investigated.

Glycylglycine,



The pancreatin has no appreciable action. On adding β -naphthalene sulphochloride to the digestion products, pure β -naphthalene sulphoglycylglycine was obtained, without any admixture of β -naphthalene sulphoglycocol.

β -naphthalene sulpho-d-alanyl-glycine,



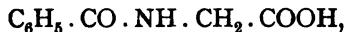
On digestion no β -naphthalene sulphoalanine was obtained, but only the unchanged original product; this body, therefore, does not react to trypsin.

β -naphthalene sulphoglycyl-d-alanine,



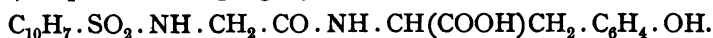
No reaction to trypsin.

Hippuric acid,



gives also no reaction to trypsin.

β -naphthalene sulphoglycyl-l-tyrosine,



This body on treatment with trypsin readily hydrolyses into tyrosine and β -naphthalene sulphoglycine.

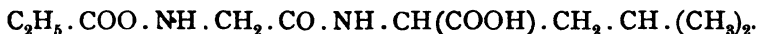
* Fischer and Bergell have shown that β -naphthalene sulphochloride $\text{C}_{10}\text{H}_7\text{SO}_2\text{Cl}$ readily reacts with amino-acids to give products of the type, $\text{C}_{10}\text{H}_7\text{SO}_2 \cdot \text{NH} \cdot \text{CHR} \cdot \text{COOH}$; by means of this reaction amino-acids can be readily isolated and identified.

β-naphthalene sulphoglycyl-d-l-leucine,



Trypsine has no very appreciable action on this body.

Carbethoxylglycyl-d-l-leucine,



This body on treatment with trypsin yields *L*-leucine isolated in the form of its naphthalene sulphochloride, and also apparently carboxyethylglycyl-*d*-leucine, and carboxyethyl-glycocoll.

Carboxyethylglycyl-tyrosine on treatment with pancreatin readily undergoes scission, giving carboxyethyl glycocoll and tyrosine.

Glycyl L-tyrosine on treatment with trypsin readily yields *L*-tyrosine and glycocoll.

Leucyl-d-l-alanine gives on treatment with trypsin leucyl *d*-alanine, and probably leucyl-*L*-alanine.

Alanyl-leucine and *leucyl-leucine* (racemic bodies) can each be resolved into an optically active dipeptide and two amino-acids. In the last three cases hydrolysis is very incomplete.

It will be observed, therefore, that the tryptic enzyme has a special predilection for certain groups, notably tyrosine; the ease with which this body, as well as leucine, is detected during tryptic digestion is due not merely to the fact that not only is it readily isolated, but it is also readily eliminated from an albumen molecule by means of the tryptic enzyme.

It is of interest at this point to note the similarity between the proteoclastic and other enzymes. Fischer has long ago called attention to the fact that certain glycoclastic enzymes have a specific action only for certain carbohydrate groups; the same kind of specificity exists also in the case of the proteoclastic enzymes; for this reason great precautions must be taken in drawing conclusions as to the structure of an albumen-like body from the order in which scission of amino-acid groups takes place when that body is treated with enzymes; this factor depends, as we have seen, on the specific action of the enzyme employed.

Summary.

We may now venture to summarise our knowledge of the degradation products of the albumens.

The Ultimate Degradation Products.—Of these products it has already been stated that about 70 per cent. have been isolated in the case of most of the albumens which have been investigated. It must be remembered, however, that the methods available are by no means quantitative.

This 70 per cent. of the degradation products includes only nitrogenous products of the amino-acid types; in addition to these, we know that carbohydrate groups have been obtained in quite a large quantity (*vide* p. 26) from several albumens, such as egg-albumen and serum-albumen. It is possible that such carbohydrate groups constitute a large part of the remaining 30 per cent. of the degradation products. Our methods for isolating these groups are up to the present very crude, and until better ones are elaborated we can hardly hope to gain much more accurate information on this subject.

There is another degradation product, the origin of which is still very obscure, viz., ammonia, which occurs always amongst the products of hydrolysis, and which can be eliminated by treatment of the hydrolysis mixture with magnesium oxide. The nitrogen contained in this ammonia has been designated "amide nitrogen"; it has formed the subject of numerous investigations, amongst which may be mentioned those of Haussmann. Its biological and chemical significance is still unknown.

The Intermediary Degradation Products.—Although the greater number of the ultimate degradation products have been isolated, we know, so far, very little about the way in which they are coupled together in the albumen molecule. A general summary of the methods now available for their investigation has been just given; these may be divided into two classes, viz., (i) the method of direct isolation of such products by degradation of natural albumens; (ii) the method of the synthetical production of the polypeptides. This latter has yielded and is yielding valuable results in the hands of E. Fischer and his pupils.

Many researches have been carried out with the object of isolating simpler peptides, amongst which may be mentioned those of Siegfried on the so-called kyrines and antipeptones, and those of Fischer on proline derivatives resistant to trypsin action, and certain peptides from serine.*

In addition to these, numerous researches have been recently devoted to the isolation of the more complex degradation products of the albumose and peptone class, especially in the laboratory of Hofmeister. Each of the individual albumoses and peptones isolated needs a complete study; we want to know not only the ultimate, but also the intermediary degradation product of each; furthermore, it will be necessary to study with the same detail also these intermediary degradation products. It is therefore evident, that in spite of the research methods available, an immense amount of work remains to be done before we can acquire anything like a complete knowledge of the structure of the albumens. It seems safe to predict that many years must elapse before this knowledge is acquired, even supposing that the methods of research now available are sufficient for the elucidation of the problem.

REFERENCES—LECTURE VIII

- A. On the Separation of the Products of Digestion by Salts :—
 PICK.—*H.Z.*, 24, p. 246, 1897; 28, p. 219, 1899; *Hofmeister's Beiträge*, 2, p. 481, 1902.
 ZUNZ.—*H.Z.*, 28, p. 132, 1899; *Hofmeister's Beiträge*, 2, p. 435, 1902.
- B. On the Isolation of the Intermediary Products of Albumen Degradation :—
 SIEGFRIED.—*Ber.*, 33, p. 2852, 1900; *Proceedings of the Saxon Academy*, p. 63, 1903; *H.Z.*, 27, p. 333, 1899; *H.Z.*, 35, p. 187, 1902.
- C. On the Synthesis of the Polypeptides :—
 E. FISCHER and Pupils.—*Ber.*, 34, p. 2868, 1901; *Ber.*, 35, p. 1095, 1902; *Ber.*, 36, p. 2094, p. 2106, p. 2592, and p. 2982, 1903; *Ber.*, 37, p. 2486, p. 2842, p. 3062, p. 3071, p. 3103, p. 3306, p. 4575, and p. 4585, 1904; *Ber.*, 38, p. 605, 1905.

* The work on serine has not yet been completely published.

LECTURES IX AND X

GENERAL THEORIES OF BIOCHEMICAL ACTION

WE have already compared the albumen molecule in the living body to a complex machine performing certain functions, which could not be removed from its working position without damaging certain of its parts. We have examined, to continue the simile, the damaged machine, and demonstrated the methods hitherto employed for identifying the parts of which it is composed.

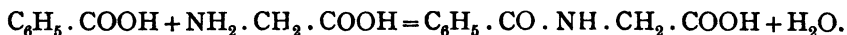
We now come to the consideration of a far more complex problem, of which up to the present very little is known, viz., the problem of the method by which the machine acts *in situ*, and performs its functions when constituting a part of the living organism.

Three facts are immediately obvious to us when we come to consider the chemistry of the living organism. These are the following :—

I. *That the organism is in a state of constant change.* To take one obvious example of this statement: oxygen is being constantly inhaled and utilised for the combustion of carbon and nitrogen compounds, which are eliminated from the organism in the form of carbonic acid, urea, etc. ; unless the loss due to this combustion in the animal body is replaced by food-stuff, the organism will ultimately cease to work, and in time dies ; a change of some sort then seems to take place in the chemistry of the albumen, and it ceases to act in the same way as it does in the living organism. This change will be discussed below.

II. *That the molecule of the albumen, which performs the normal chemical functions necessary for the maintenance of life is, compared with ordinary molecules with which we are in the habit of dealing in the chemical laboratory, of enormous size, and very unstable; it loses many of its properties on the application of a very moderate amount of heat.*

III. *The chemical reactions necessary for the maintenance of life take place within very narrow limits of temperature.* When we wish to bring about a chemical reaction of any description in the laboratory, we have at our disposal a variety of methods for varying the conditions of experiment. In order that chemical reaction should take place between different molecules, it is necessary that they should come into frequent contact with one another. This result can be brought about in the laboratory by using high temperatures, and in some cases high temperatures combined with increased pressures. Now attention has already been called to the fact that the properties of an albumen molecule are readily destroyed by the application of a very moderate amount of heat; furthermore, all reactions in the animal body take place at a temperature not far removed from 37° C. As examples of such changes we may mention the oxidation of fats and carbohydrates by means of the oxygen of the air; this is an example of a degradation change. As an example of a synthetic reaction we may mention the formation of hippuric acid by the condensation of benzoic acid with glycoll,



Now neither of these changes can be accomplished readily *in vitro* at the temperature of 37°.

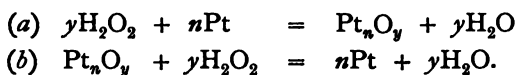
Hence we are dealing with abnormally large molecules which are constantly bringing about chemical change at a temperature of about 37° C., which changes occur *in vitro* only at higher temperatures, and with the use of energetic chemical reagents which do not exist in the body.

We are forced, therefore, to seek for some special explanation of biochemical reaction.

Some reactions of the albumens which we have already studied give us a preliminary indication of the direction in which we may seek a possible explanation of such biochemical action.

We have seen that the addition of minute amounts of gastric or pancreatic juices is capable of promoting the hydrolysis of large amounts of albumen; this reaction of the juices takes place, furthermore, at temperatures at which the amount of hydrolysis by acids and alkalies alone is extremely small. The substance contained in the juice, except in so far that it is itself slowly destroyed by water at the temperature of reaction, remains practically unchanged after it has caused the hydrolysis of a relatively very large amount of albumen. We have in these cases an instance of the so-called *catalytic action*.*

In recent years, the number of substances obtained from both plants and animals which can act as catalysors has increased enormously; their reaction is confined not only to processes of hydrolysis, but different catalysors increasing the rate of oxidation, and specific for the oxidation of certain substances or classes of substances only (*cf.* Jacoby on the Oxidases of the Liver, *Ergebnisse der Physiologie Biochem. Abth.*, vol. i.) have been isolated from different organs of plants and animals. Analogous reactions are known to take place with inorganic products, *e.g.*, the decomposition of hydrogen peroxide by means of platinum black. This reaction has been explained by Bredig by means of the following equations:—



Here we see that hydrogen peroxide is decomposed with evolution of oxygen, whilst the platinum remains unchanged at the end of the reaction. The equations given assume the formation of a labile intermediary addition product of the catalysor; it is probable that in the more complex enzymes of organic origin similar addition products would be formed.

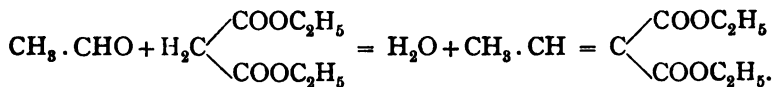
* "A catalysor is a body, which, without appearing as an end-product in a chemical reaction, alters its velocity."—OSTWALD.

Now let us consider the case of an albumen molecule undergoing degradation by means of a proteoclastic enzyme. The molecule of the albumen is, compared with an ordinary molecule, already enormously large, and probably has but a very small mean free path.

The formation of an addition product with an enzyme (which is probably also a body of great molecular weight, would still more increase the space extension (German, "Raumerfüllung") of such a molecule, and build it up to such an extent, that we might expect that it would readily come into contact with other molecules or ions (in the case of proteoclastic enzymes of water) and then undergo decomposition with the liberation of the enzyme in an unchanged form, which would then be at liberty to combine with other molecules of an albumen, which in their turn would undergo hydrolysis. In this way we should obtain the same effect by means of catalysors as is obtainable in other cases by increase of the temperature of reaction.

So far, only such catalysors have been obtained from living bodies which are capable of accelerating degradation processes. We know, however, that synthetical processes can also take place in an organism; catalysors capable of accelerating a synthesis have, so far, not yet been isolated. There is, however, no reason why such bodies should not exist, and though the more complex synthetic catalysors remain still undiscovered, it is of interest to remark here that a simple catalysor bringing about a relatively simple synthetic reaction is already known.* Aldehydes are capable of acting on ethylmalonate and allied substances at high temperatures in presence of strong dehydrating agents like acetic anhydride, *e.g.*:—

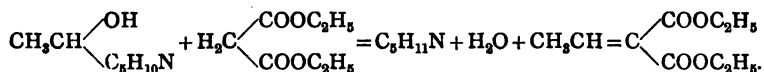
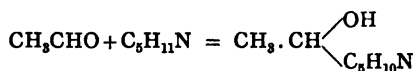
CLAISEN.



Knoevenagel has found, however, that this reaction can take place at temperatures as low as -15°C . in the presence of minute

* Schryver, *Proceedings of the Physiological Society*, January 1904.

quantities of piperidine and other bases. He explains the reaction by means of the following equations:—



We have here possibly an extension of the sphere of action of the carbon atom of the aldehyde group by the formation of an intermediary unstable addition product with the base. Thus there is a simple example of a catalysor accelerating a synthetic reaction. The conception of the formation of such labile intermediary addition products by the bioplasm with various groups forms the bases of the newest hypothesis of biochemical action; but before proceeding to the statement and the discussion of this newest theory of Verworn, it will be advantageous to pass in rapid review some of the various hypotheses which have existed from time to time on this subject.

Hermann's Hypothesis, 1867.—The first biochemical hypothesis, which was enunciated by Hermann, refers almost exclusively to muscular action.

Hermann found that if an extirpated muscle from which all the oxygen had been pumped out be allowed to work in an oxygen-free atmosphere, carbonic acid and a non-volatile acid (afterwards identified as sarcolactic acid) were produced. He found that at the same time myosine in a gelatinous form separated; he thus early recognised the similarity of the phenomena of rigor and exhaustion. If blood be added before coagulation was complete, the myosinè redissolved. This restitution of muscle Hermann described as a synthetic process, and he described the whole phenomena as follows:—

The degradation of a nitrogenous body is the essential phenomena of muscular action. Amongst the degradation products identified, viz., carbonic acid, non-volatile acid, and the gelatinous albuminous body, the last named at least, if not also the non-volatile acid, takes part in the restitution of the

molecule, and it is only the carbonic acid which is known with certainty to leave the molecule.

Here we have for the first time stated in a perfectly definite form a theory of degradation and subsequent restitutive synthesis of a complex nitrogenous compound. The molecule in its more complex form can combine with oxygen, which is thereby held at the disposal of the organism for the purpose of oxidation. The albumen molecule acts more or less as an oxidase.

The Hypothesis of Pflüger, 1875.—Pflüger observed that a frog was capable of living for twenty-five hours in an oxygen-free atmosphere at the temperature of a few degrees above zero, and of exhaling carbonic acid gas during the whole period. He, too, came to the conclusion that the albumen in the living organisation is capable of combining with the oxygen, and holding it at the disposition of the organism. He assumed the existence of two factors in biochemical action, viz., (1) the capacity of the "living albumen molecule" to combine with oxygen ("intra-molecular oxygen"); and (2) the existence in the "living molecule" as opposed to the dead molecule, of certain groups, viz., the cyanogen groups, the presence of which added to the stability of the molecule. It is the presence of the cyanogen group, according to Pflüger, which distinguishes the living from the dead molecule. The ground for the assumption of the presence of cyanogen groups is the fact that the nitrogen decomposition products obtainable from living matter, such as uric acid, creatine, urea, either themselves contain a cyanogen group, or can be readily prepared from bodies containing such a group. On the other hand, according to Pflüger, "dead albumens" on degradative oxidisation never yield these products. This latter statement is now known to be incorrect; we shall, furthermore, presently learn that it is possible for urea to be derived by means of quite a different chemical process. Pflüger assumed that addition of oxygen took place to the cyanogen carbon, which is subsequently eliminated in the form of carbonic acid. We have then a degradation of the molecule. In addition to this, the possibility of a growth of the albumen molecule is also

assumed ; this phenomenon takes place by means of polymerisation, a phenomenon which often occurs in a large number of simple organic compounds, especially those containing a cyanogen group. In this way, according to Pflüger, gigantic molecules can be formed ; these can combine with oxygen, and when necessary undergo degradation by means of the process already described.

Pflüger summarises his hypothesis as follows :—

“The process of life is the intramolecular heat of extremely unstable albumen molecules of the cell substance, which are continually decomposing by dissociation with the formation of water, carbonic acid, and amino-bodies. The dissociated molecules are being continually regenerated by means of polymerisation.”

It might be added that there is so far no evidence to support Pflüger's assumption of the existence of cyanogen radicals in the “living albumen molecule.”

Hypotheses similar to that of Pflüger have been advanced by the botanist Detmer (1880), Loew (1880-89), and F. J. Allen (1899). These need no special discussion in this place.

The Hypothesis of Ehrlich, 1885.—We now come to consider an important conception advanced first by Ehrlich in 1885, which has recently played a very important rôle in biological thought. This is the so-called “side-chain” theory.

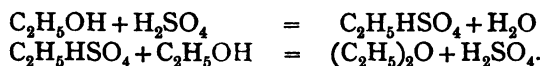
The main principle of this theory, as far as it applies to normal physiological processes, may be stated in a very few words. Certain parts of the acting complex are capable of anchoring certain molecular groups, which can be readily oxidised ; other parts of the molecule are capable of anchoring oxygen. The recombination of oxygen and the renewal of the oxidisable side-chain are both possible.

In this conception, like in those of Pflüger and Hermann, the assumption is made of the possibility of the formation of intermediary addition products, which can readily dissociate. The whole molecule is conceived as having an enzyme-like action. The theory of Ehrlich contains, however, an entirely new idea, viz., the specificity of action of different parts of the

molecule ; certain points only are capable of anchoring certain specific groups.

Now when we come to remember how extremely specific certain enzymes are in their action, how, *e.g.*, certain sugars will undergo fermentation only in the presence of certain species of yeast, and how glucosides can be decomposed only by certain specific enzymes, the importance of Ehrlich's generalisation is manifest. If we assume with E. Fischer that the enzyme fits the body for which it is specific in the same way that the key fits the lock, we shall have to assume a definite peripheral configuration for an albumen molecule, when performing its function in the living organism ; and we shall have to take into account the consequences of a definite stereochemical configuration.

The formation of intermediary addition products with groups such as nutritive side-chains or oxygen, and the subsequent scission in the living organism may be compared to a continuous chemical process, as, *e.g.*, the formation of ether.



In this case the alcohol is allowed to flow continually into the sulphuric acid contained in the distillation flask, and the ether as it is formed distils off by a side-tube. The alcohol stream may be compared to the stream of arterial blood carrying the metabolites to the tissue, whilst the ether stream may be compared to the venous blood carrying away the katabolites. The tissue is in a state of continuous change like the sulphuric acid in the ether process, continually forming addition products which are subsequently decomposed.

We now pass to the consideration of the latest biochemical hypothesis.

The "Biogen" Hypothesis of Verworn, 1903 (Jena : Gustav Fischer, 1903).—Verworn in his new hypothesis combines the conception of the labile oxygen compounds with the Ehrlich theory of side-chains. We owe to him, in the first instance, the introduction of a new term. Instead of using such illogical expressions as "living albumen molecule," he proposes to term

the molecules in the living protoplasm performing their normal functions for the maintenance of life the "*biogen molecules*." His hypothesis as to their action he calls the "*biogen hypothesis*."

He assumes that, in addition to the ordinary nitrogenous groups (derived from the condensation of the amino-acids in the way already described) there exist other "side-chains" in the molecule of a carbohydrate nature, which can be readily oxidised by the "intramolecular" oxygen, with the formation of carbonic acid. Such an oxidation would readily take place if we were to assume that these carbohydrate groups contained an aldehyde complex. Verworn assumes that these carbohydrate side-chains are attached to a benzene nucleus. The "intramolecular" oxygen would be attached to a nitrogenous group, and after oxidation a restitution of carbohydrate and oxygen would take place. The carbonic acid would be carried away as a katabolite, and the restitution would take place from the food-stuff material continually carried to the tissue; the whole mechanism would be analogous then to a continuous chemical process, of which an example, viz., the preparation of ether, has been already cited. The example cited by Verworn is that of the manufacture of sulphuric acid; in the case of the biogen molecule, according to the hypothesis, the nitrogenous group acts as the oxygen carrier, whereas in the manufacture of sulphuric acid the nitric oxide performs this function. The following are the main points of the theory in Verworn's own words.

(i) The functional dissociation of the biogen molecule depends on the fact that the intramolecular oxygen is transferred from its position of anchorage to an easily oxidisable nitrogen-free side-chain; the carbon is thereby oxidised to carbonic acid, with the further possibility, in the case of a more complete oxidation of a side-chain, of the formation of other nitrogen-free degradation products such as lactic acid and water.

(ii) If one wishes to construct a schematic model of a biogen molecule, one can assume that the oxygen receptor and translator is a nitrogenous or iron-containing group,* and the material

* As it is known that various nitrogen and iron compounds are "carriers" of oxygen.

for oxidation a carbon chain of the carbohydrate type, with an aldehyde group, both groups, *i.e.*, the nitrogenous or iron-containing oxygen receptor, and the combustible carbohydrate chain being attached to a benzene ring.

With regard to the regeneration of the molecule, Verworn assumes the following conditions :—

(i) The valencies set free by the functional dissociation of the biogen molecule can be satisfied by suitable carbohydrate groups and oxygen, and the *status quo ante* thereby restored.

(ii) These groups necessary for restitution can be supplied by the products prepared in the normal processes of digestion.

(iii) Under normal circumstances the dissociation and restitution take place with equal velocity. The idea of equal and opposite “assimilation” and “dissimilation” was due originally to Hering.

(iv) Want of oxygen and available combustible material after complete utilisation causes, not a condition of “latent life,” but brings about a more radical decomposition of the biogen molecule, with an accompanying loss of restitutive power. The conditions regulating tissue stability will be discussed later. There still remains the fate of the nitrogenous matter to be considered, for we know that albuminous food is eliminated from the organism principally in the form of uric acid and urea.

With regard to the fate of the nitrogenous matter, Verworn makes the following assumptions :—

(i) In metabolism two kinds of biogen degradation exist, *viz.*, the “functional” and the “destructive.”* The former is of the nature of a dissociation.

(ii) Functional dissociation is concerned only with the nitrogen-free degradation products (this action has been already discussed). It rises or sinks in accordance with the needs of the organism.

(iii) Destructive degradation consists of a radical decomposition of the biogen molecule with the elimination of nitrogenous groups. It is secondary in importance to the functional dissociation, and is to a great extent independent of

* Cf. Folin, *American Journal of Physiology*, 1905.

the functional needs of the living substance, and exists only in consequence of the great lability of the biogen molecule, which in consequence of the processes continually taking place in the organism must from time to time undergo some alteration.

We have, then, according to this theory, a certain loss of biogen molecules, and Verworn assumes with Pflüger that a "living molecule" is capable of taking up fresh material and growing; "giant molecules" are thereby built up, which, on attaining a certain size, break down into simple biogen molecules. This process is analogous to reproduction by scission of certain simpler organisms.

Verworn thinks that the biogen is contained only in the protoplasm of the cells, and that the cell nucleus forms a depôt of material necessary for restitutive processes.

There is one other point to which reference must be made, viz., the source of material for restitution. Verworn considers that this material is of quite simple character, and that it is obtainable directly from the food-stuffs by the action of the various digestive fluids. When this source of supply fails, the organism can draw upon the reserve depôts of the cell. As an instance of such reserve depôts we may mention the glycogen, which, as we know, very rapidly disappears in cases of hunger. Verworn also suggests that certain organised protoplasm can serve as store material, and as an example of a reserve of this kind he cites the Nissl granules of the nerve cells. Gordon Holms, working in Verworn's laboratory, showed that ganglion cells dying of acute starvation did not show the characteristic tigroid bodies described by Nissl. Furthermore, in cases of acute need, the biogen molecules themselves can undergo degradation, and the simple products thereby prepared* can serve as restitutive material for the still intact biogen molecules. One set of molecules is thereby retained in activity at the expense of another set.

We have, then, according to Verworn, three sources of nutritive material necessary for the restitution of the biogen molecule—(i) the nutritive material derivable directly from the

* This phenomenon will be discussed later.

food-stuff; (ii) the reserve protoplasm in the organism; (iii) failing these, the biogen molecules themselves.

We come now to a criticism of the theory of Verworn.

The conception of labile intermediary products formed by the addition of different elements or groups to different side-chains is a useful one. According to the idea here expressed, the whole biogen molecule acts as a collection of enzymes, and there is much experimental evidence to support this view.

There are, however, several details in the Verworn hypothesis to which objections must be raised, and it will be necessary to consider a few of these in this place.

Firstly, there is the conception that the nucleus to which the side-chains are attached is a benzene ring. There is very little evidence to support such a theory; on the contrary, there are several reasons which render the benzene-nucleus hypothesis extremely unlikely.

(i) The molecule of an albumen is very complex; we must assume, therefore, as there are only six points in a benzene ring to which a side-chain can be attached, that there can be only six, at the most, side-chains in an albumen molecule. Each side-chain must therefore be a very long one. Now, if each be attached to a benzene nucleus, whether we adopt the old Kekulé formula for benzene, or the centric formula, the side-chains will tend to spread out away from one another in space. We shall then have just such an arrangement that will be least favourable for any kind of contact action between the side-chains.

(ii) The simplest albumen molecules, viz., the protamines of certain spermatozoa, it has been shown by Kossel, contain, as far as we know, no benzene groups, and certainly more than 80 per cent. arginine. These protamine groups form a nucleus on to which other groups can be built, and the idea of Kossel, that the hexone base complex is a nucleus around which the albumen molecule is developed, has certainly a great deal more probability about it than the benzene-nucleus conception of Verworn.

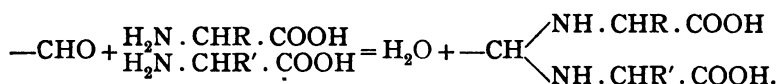
(iii) The scission of tyrosine, the most important benzene

body derivable from the albumen, takes place more readily than that of almost any other amino-acid. Not only does this happen when natural albumens are treated with enzymes, but also, as E. Fischer has shown (see p. 164), when the synthetical peptides are treated in the same way. This we should hardly expect if the tyrosine contained the benzene nucleus to which the side-chains are attached, as Verworn suggests.

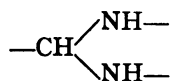
The question of the nucleus to which the side-chains are attached, is, however, a minor point; an erroneous speculation on this point need not detract appreciably from the value of the main hypothesis.

We next come to a criticism of Verworn's conception as to the fate of the nitrogen bodies in metabolism.

It seems quite unnecessary to assume that the so-called "functional" dissociation concerns only the oxygen and carbohydrate side-chains. If we assume with Verworn that certain carbohydrate-like side-chains exist with a terminal aldehyde group, it is quite conceivable that such a group can enter into combination with nitrogenous degradation products of the albumenous food-stuffs according to the following equation:—



We should by such a condensation obtain a side-chain with the complex



with the nitrogen attached to secondary carbon atoms. A grouping of this sort would readily oxidise to urea.*

After such an oxidation we should imagine a restitution of the carbohydrate side-chain, recondensation with amino-bodies, and oxidation of the complex thus formed. The adoption of such a theory renders it quite unnecessary to assume a "de-

* Experiments have been commenced by the author with the object of verifying this theory.

structive" degradation of the biogen molecule to account for the nitrogenous metabolism.

Furthermore, there is physiological evidence in favour of the view that nitrogenous matter can be directly utilised as a source of energy.* Pflüger has shown that a dog can be maintained in perfect health whilst performing arduous work on an almost purely albuminous diet. An athlete can thrive during training on a large albumen diet. If the theory of Verworn be correct, we should assume that the most economical diet would be one rich in carbohydrates, and the energy would then be derivable entirely from functional dissociation. It is true, as Seegen has pointed out, that a large amount of carbohydrate is derivable from albumens by their degradation; even in this case, however, if Verworn's ideas are correct, the albumen could not afford the most economical source of supply for muscular energy if the latter be derived from "functional" dissociation. Furthermore, if the energy during muscular work be derived from "destructive" degradation, as is possible from Verworn's theory, we should observe a difference in chemical stability between acting and resting muscle. Such a difference does not, however, exist.†

The consideration of these points in detail will, however, lead us into a discussion of several points in metabolism; we must content ourselves at this point, therefore, with this very incomplete criticism.

We proceed now to discuss Verworn's ideas as to the sources of restitutive material. Here we are fortunately able to support some of his views by experimental facts.

* See, however, succeeding footnote, quoting Chittenden's work.

† Researches of the author, not yet published. It is interesting to note that Chittenden has recently shown that equilibrium, even during strenuous physical work, can be maintained with a very small albumen diet. It is also possible that, as Folin has recently suggested, a considerable part of the nitrogen from the products of tryptic digestion is rapidly eliminated in the form of ammonia (and subsequently converted into urea), and that the organism utilises only the carbohydrate residue as a source of energy. The author hopes to discuss, however, the function of nitrogen in a future publication.

Verworn assumes that the first source of restitutive material is the food-stuff offered to the protoplasm, in the case of nitrogenous material at any rate, in a very simple form. Failing this, the organism draws upon its food-reserve in the protoplasm, and failing this second source, upon the bioplasm itself. He rightly assumes that the mechanism is regulated by enzyme action. He draws attention to various mechanisms existing in plants for the regulation of the supply of food material. The most interesting case he cites is that of the cellulose-splitting enzyme in the scutellum of germinating barley. This enzyme only comes into play when there is a dearth of simple carbohydrate, the normal food-stuff of the organism. As soon as this carbohydrate has been utilised, and failing any fresh supply, a cellulose-splitting enzyme is developed in the scutellum; the organism uses thereby its own tissue as a source of food.

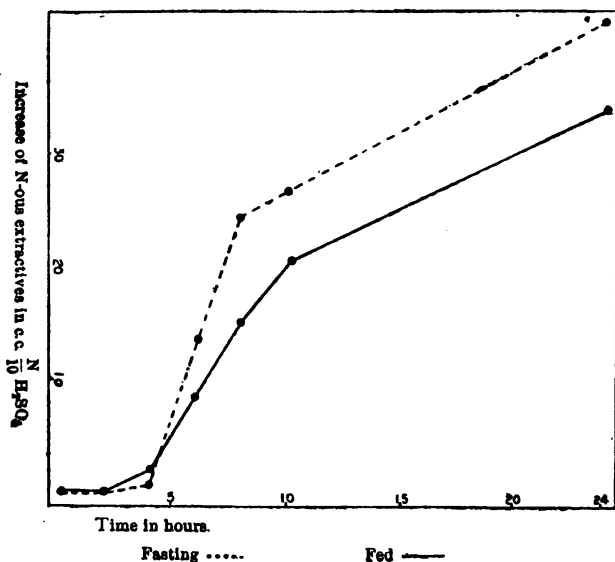
An exactly analogous mechanism exists in the animal body. Miss J. E. Lane-Claypon* and the author have recently shown that the proteoclastic ferment which was discovered by Sal-kowski and called by him the autolytic enzyme, acts more rapidly when the animal is fasting than when it is well nourished. The accompanying figure (p. 182) illustrates the rate of action of this autolytic enzyme on the livers of two animals, one of which was killed after a short period of starvation, and the other shortly after a meal. It will be observed that the albumen of the fasting animal degrades at an appreciably greater rate than does that of the well-fed.

It will be noticed that before degradation sets in, there is a "latent period," lasting about four hours, during which no diminution of the amount of coagulable albumen takes place. In one case, when an animal has fasted twice during a week, it was observed that this latent period is absent. We might assume in this case that the autolytic enzyme is acting during life.

In the last paper on this subject, it has been suggested that the chemical stability of a tissue at any particular moment is the resultant of the mass action of three different sets of sub-

* Lane-Claypon and Schryver, *Journal of Physiology*, 31, p. 169, 1904; and Schryver, *ibid.*, 32, p. 159, 1905.

stances, viz.: (i) the tissues themselves; (ii) the autolytic ferment, either in enzyme or zymogen form; (iii) a series of bodies, either metabolites themselves, or prepared for metabolites. As long as the bodies of class (iii) are present in excess, the autolytic enzyme will not act on the tissues. These bodies are apparently easily destroyed. (Possibly they take part in the "functional" dissociation of the tissue.) As long, however, as excess of food-stuff is present in normal conditions,



they can be re-formed. In the event, however, of a dearth of bodies of this class, the autolytic enzyme acts on the tissues, which then form a source of food-material for the organism. We see, then, that the autolytic enzyme performs an important function in the organism, and serves to protect the animal against starvation. The nature of the inhibitory bodies and their mode of formation forms now the subject of a research.

One point of further interest may be noted here, viz., the resistance of the albumen in the living organism to degradation. This suggests the possibility that the albumen functioning

in the living organism always tends to maintain its maximum size and spacial extension. It is possible, therefore, that the characteristic of the albumen in the living body as opposed to that in the dead, is that the former has attained a greater growth and spacial extension as compared with the latter. If we adopt this view, then the side-chains in the different molecules will approach near one another in the living mass, and the chances of contact of any material present in a tissue, such as a metabolite, with any particular side-chain is thereby increased. Hence the greater possibility of chemical action in the very complex molecule of the living tissue, as compared with the less complex molecule in the dead.

Our knowledge of the action of the albumens in the living organism is, so far, very limited. We have many theories and comparatively few facts. If the conception of the former, however, stimulate the acquisition of the latter, they will justify their existence.

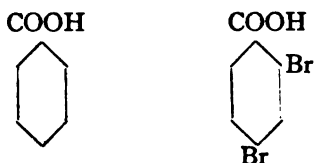
Of the theory of Verworn, which we have discussed in some detail, the most we can say at the present moment is, that in its essentials it forms a fair working hypothesis.

Conclusions.—It will be seen from the above considerations, that there is a large field of work open to investigators on the question of the chemical dynamics of the living tissue. This is a subject to which our knowledge of the influence of chemical mass-action must be applied—a subject which has engaged the attention of many distinguished investigators for several decades.

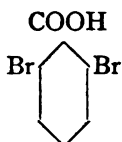
In addition, we owe to recent chemical research various other new conceptions, which are capable of application to biological chemistry. The influence of the surrounding medium on the chemical configuration we have already discussed, when considering the pseudo-acid and pseudo-base-like character of the albumen molecule. One other chemical factor to which recent research has drawn attention may be mentioned here, viz., the influence of stereochemical configuration on chemical action.

Victor Meyer has shown that benzoic acids can be readily

esterified by alcohol containing 3 per cent. hydrochloric acid. If, however, the benzene ring be substituted in two positions ortho to the carboxyl group, esterification will not take place with this reagent.

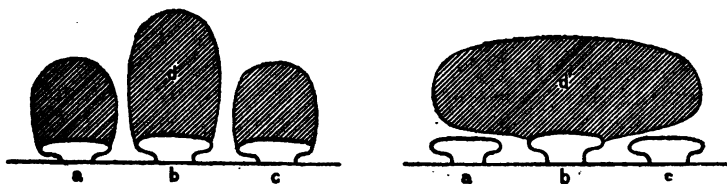


Benzoic acid and 2,4-dibrom-benzoic acid are readily esterified by alcohol containing hydrochloric acid.



1,5-dibrom-benzoic acid resists esterification with the same treatment. Victor Meyer ascribes the non-reactivity of the diortho-body to the stereochemical inhibition of the two bromine groups in the ortho-position. The proximity of these two large atoms, occupying relatively much space, inhibits the normal action of a carboxyl group.

It is quite possible that we may have similar stereochemical inhibition of the normal action in the albumens in living tissue. If we assume the presence of side-chains near to one another, it is conceivable that one side-chain may anchor at some time or



other a different group to that which it usually attaches, which may inhibit the normal action of the neighbouring side-chain.

Supposing the side-chains be graphically represented above as a , b , c , to which certain groups (shaded) anchor themselves. Suppose d is a normal group, which attaches itself to b . Under normal circumstances a and c will attach groups to themselves and perform their ordinary function. Suppose, instead of a normal group d , an abnormal group d' , occupying much space, should attach itself to b . It is conceivable that this group will inhibit the normal haptophore functions of a and c , just as the bromine atoms inhibit the esterification of the carboxyl group in the benzoic acids.

We can, therefore, conceive toxicity in a tissue as due to a stereochemical inhibition of normal functions.

By a mechanism of this sort we can conceive the stereochemical adaptation of the periphery of molecule to different circumstances, and the possibility of the formation under these circumstances of different side-chains, which can be thrown off, according to Ehrlich's immunisation theory, and form antitoxins.

This is obviously a subject which cannot be discussed in detail here. The remarks just made will suffice to call attention to an important chemical factor, which has so far been neglected in biochemical considerations.

Biological chemistry may be studied, it will be seen, from two different aspects. We may either devote our attention to the elucidation of the structure of the albumen molecule, or we may investigate the actions of the molecule when performing its normal functions in the living animal.

An attempt has been made in these lectures to summarise our knowledge of the constitution of albumens at the present time; as to the action of the albumen in the living molecule, little more than a crude sketch and criticism of the various theories has been given.

It is hardly necessary to dwell upon the importance of a knowledge of albumen chemistry and its manifold applications to physiological and pathological research. Most of our knowledge is of recent acquisition, and hardly sufficient time has elapsed for us to see great progress in its application; little is

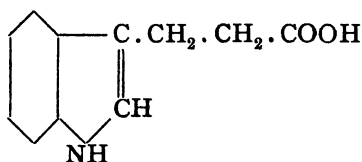
known, for example, of the chemical differences between tissues in health and disease.

Our progress, however, in the elaboration of methods has been very rapid during recent years, and it is not too much to hope that chemistry in the immediate future will play a far more important part than it has in the past in the development of biological research.

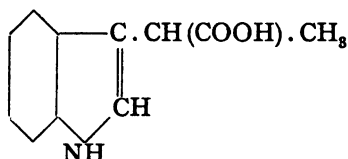
APPENDIX

A. TRYPTOPHANE.

ELLINGER has recently succeeded in showing that the supposed skatol-acetic acid of Nencki is in reality indol-propionic acid of the formula—

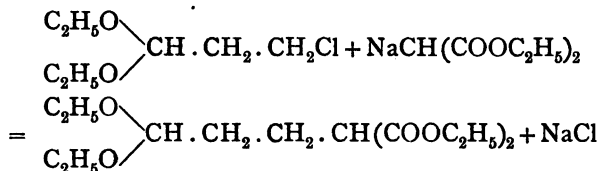


and not

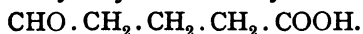


as it would be if the formula assigned by him to tryptophane (as given in the text) be correct.

He has synthesised the body in the following way:— β -chlor-propionacetyl is treated with ethyl sodio-malonate, and the product thus obtained is hydrolysed. The semi-aldehyde of glutaric acid is thereby obtained. The phenylhydrazide of an ester of this body, on treatment with alcoholic sulphuric acid, is converted into the ester of indol-propionic acid, which is identical in properties with the Nencki acid obtainable from albumens. Formulæ:—

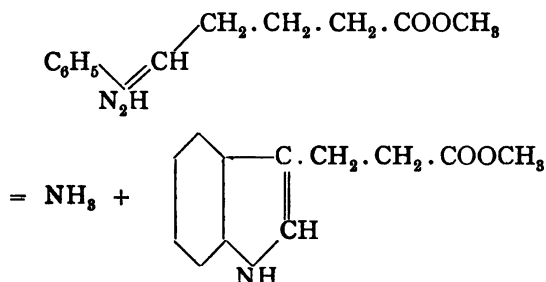


This acetal yields on hydrolysis the aldehyde



The phenylhydrazide of the methyl ester undergoes conversion

on treatment with alcoholic sulphuric acid according to the following scheme :—

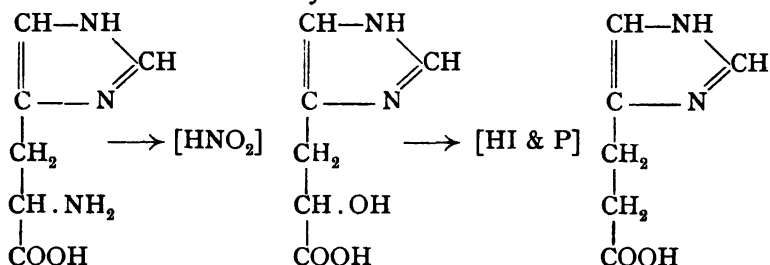


(Ber., 38, 2884, 1905).

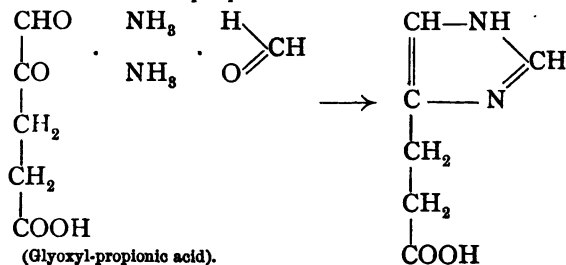
B. HISTIDINE.

The formula given in the text as most probable for this body is supported by some recent researches of Knoop and Windaus.

On treatment with nitrous acid, the amino-group in histidine is replaced by a hydroxyl group. This latter group can be replaced by hydrogen on treatment of the body containing it with phosphorus and hydriodic acid. The body thereby obtained (imidazol-propionic acid) has been synthesised by Knoop and Windaus, by treating glyoxyl-propionic acid (Wolf-Liebig's *Annalen*, 260) with ammonia and formic aldehyde. Formulæ:—



Synthesis of imidazol-propionic acid.



(Hofmeister's *Beiträge*, 7, 1905.)

INDEX

- ACETHÆMINE**, 126
Acrylic acid, 100
Adamkiewicz reaction, 10, 36
Adenine, 82, 95
Alanine, 21, 31, 47
Albumens, crystallised, 14-17
 reactions of, 1-10
Albumoses, separation of, 10-14, 140
 et seq.
Aldehydes, condensations of, 170-171
Alkachlorophyll, 132
Alkaloidal precipitants of albumens, 7
Allantoine, 84, 86
Alloxan, 86
Alloxantine, 84, 86
Alloxuric acid, 84
Amino acids, 21, 22, 41-63
Amino-butyric acid, 21
Amino-hydroxy-succinic acid, 22
Amino-valerianic acid, 72
6-Amino-dichlor-purine, 12
Ammonium sulphate as precipitant,
 141
Amphoteric, character of albumens, 2
Anilino-phenyl-phosphoric acid, 107
Animal gums, 118
Antipeptones, 150-153
 α -Antipeptone, 152
 β -Antipeptone, 152
Apparatus for fractional distillation,
 46
Arabinose, 26
Arbacine, 70
Arginine, 22, 53, 56-60, 69
Aspartic acid, 22, 48
Asymmetric carbon atom, 51-53

BACTERIA, degradation by, 37-38, 57
Barbituric acid, 85, 86
Baumann-Schotten reaction, 52
Benzoyl alanine, 52

Benzoyl chloride, 52
Benzoyl-glucosamine, 25
Benzoyl-serine, 35
Bial's reaction, 9
Biogen theory, 174-183
Biuret reaction, 8
Brom-acetyl urea, 85
Brom-capronyl-glycine, 161

CADAVERINE, 23, 60
Caffeine, 93, 95
Carbohydrate reactions, 9
Carbohydrates in albumens, 23-26
Carbohydrates in nucleic acid, 80-
 81
Carbohydrates in glyco-proteids, 20
Carp testicles, 69
Cartilage, 112
Caseanic acid, 23
Caseine, 111
Caseinic acids, 23
Caseokyrine, 153
Catalytic action, 169
Cell nucleo-albumens, 111
Chitosamine, 25, 26-27
Chlor-acetyl-chloride,
Chlorophyll, 132
Chlorophyllan, 132
Chondroitic acid, 112
Chondroine, 115
Chondrosine, 115
Chromatogenic group, 20
Clupeine, 66
Cresol, 38
Crystallised albumens, 14-17
Cyanamide, 60
Cyanuric acid, 84
Cycloptertine, 72
Cysteine, 21, 27-32
Cystine, 21, 27-32, 33-35, 49
Cytosine, 97, 100-102

- DEHYDROCHLORIDE** of hæmine, 127
 Destructive degradation, 18
 Deutero-albumose, 141, 147
 Diacipiperazines, 156
 Diamino acids, 22
 Diamino-adipic acid, 22
 Diamino-caproic acid, 60
 Diamino-dihydroxy-succinic acid, 22
 Diamino-glutaric acid, 22
 Diamino-trihydroxy-dodecanic acid, 22, 50
 Diamino-valerianic acid, 57
 Diazo-benzol benzene-imidazol, 106
 Diazo-benzol xanthine, 106
 Dibrom-valeryl chloride, 160
 Dichloro-dithio-lactic acid, 29
 Di-iodopurine, 95
 Dimethylamino-benzaldehydreaction, 10

EGG albumen, crystallisation of, 14-17
 properties of, 1-17
 Ehrlich's hypothesis, 173-174
 Ehrlich's reaction, 10
 Enzymes, action on polypeptides, 162-164
 Ethyl acetoacetate, 3
 cyanopropyl-malonate, 60
 formate, 34
 formyl-hippurate, 34
 hippurate, 34
 mercapto-6-oxypyrimidine, 101
 phyllotaonin, 132
 propyl-acetoacetate, 135
 pseudo-thiurea, 101
 sodio-formylacetate, 101
 sodio-malonate, 58

FORMIC acid, 79

GADUS histone, 70
 Globin, 123-125
 Glucosamine, 9, 26-27
 Glucosaminic acid, 27
 Glutamic (Glutaminic) acid, 22, 48
 Gluten-chondrine, 113
 Glutokyrine, 154
 Glykalbumoses, 24
 Glycocoll, 21, 47
 Glycocoll ester, 156
 Glycolyl aldehyde, 32

 Glyco-proteids, 112-120
 Glycosamine, see Chitosamine, 119
 Glycuronic acid, 116
 Glycyl, 157
 Glycylglycine, and other glycyl derivatives, 157
 Glyoxalic acid, 86
 Glyoxylic acid, 10
 Goose-blood histone, 71
 Guanidine, 59, 96
 Guanidine-butyric acid, 59
 Guanine, 82, 96
 Gulose, 119

HÆMATIN, 122, 124-137
 Hæmatinic acids, 130
 Hæmatoporphyrin, 128, 130 *et seq.*
 Hæmine, 124-127
 β -Hæmine, 126
 Hæmochromogen, 122
 Hæmoglobin, 14, 20, 121-138
 Hæmopyrrol, 133 *et seq.*
 Hair, hydrolysis of, 29
 Hermann's hypothesis, 171
 Hetero-albumose, 141, 144
 Hetero-xanthine, 90
 Hexone bases, 53-61
 Histidine, 22, 54, 61, 69
 Histones, 69-71
 Hopkins and Cole's reaction, 10
 Horn, hydrolysis of, 29
 Hydantoine, 84, 85
 Hydroxy-amino acids, 22, 49
 Hydroxy- β -quinoline-carbonic acid, 40
 Hydroxy-phenyl-acetic acid, 37
 Hydroxy-phenyl-ethylamine, 23
 Hydroxy-phenyl-propionic acid, 37
 Hydroxy-pyrrolidine-carboxylic acid, 49
 Hypoxanthine, 82, 95

IMIDAZOL, 106
 Indol, 37
 Indol-acetic acid, 39
 Indol-propionic acid, 40
 Intra-molecular oxygen, 175
 Iso-cysteine, 33
 Iso-leucine, 21

KNEVENAGEL'S reaction, 170
 Kynurenic acid, 40
 Kyrines, 153-155

- LACTAM and Lactim forms, 5
 β -Lactyl-urea, 33
 Lead sulphide reaction, 8
 Leucine, 21, 47
 Leucinimide, 21, 156
 Leucyl derivatives, 160
 Liebermann's reaction, 10
 Lipochromes, 137
 Lota-histone, 71
 Lysatine, 53
 Lysine, 22, 53, 60, 69

 MALEIC anhydride, 137
 Mesoporphyrine, 129, 132
 Metallic salts, precipitants of albumens, 6
 Methyl-acrylic acid, 99
 ethyl-maleic anhydride, 131
 imide, 134
 propyl-maleic acid, 135
 uracil, 89
 Millon's reagent, 8
 Molisch-Udransky reaction, 9
 Mono-methyl-xanthine, 90
 Mucine, 118
 Mucoids, 118
 Murexide reaction, 84
 Mycomelinic acid, 84

 NAPHTHALENE sulpho-chloride, 49
 Natural albumens, 2
 Nitroethane, 3
 Nitroform, 4
 Nitro-uracilic acid, 89
 Norisosaccharic acid, 26
 Nucleines, 77
 Nucleic acid, 20, 74-112
 Nucleo-albumens, 111
 Nucleo-proteid, 20
 Nucleo-thymic acid, 79

 OPTICAL properties of degradation products, 51-53
 Optically active acids, 51-53
 Orcine reaction, 9
 Ornithine, 57, 59
 Ovarial cysts, 118
 Oxalic acid, 85
 Oxaluric acid, 84
 Oxyhæmoglobin, 61
 Oxyproline, 23
 Oxypurines, 91

 PARABANIC acid, 84, 85
 Paramucine, 118
 Paranucleic acid, 111
 Paranucleines, 111
 Paraxanthine, 95
 Peptides, 155-164
 Peptochondrine, 113
 Peptones, 140 *et seq.*
 Pflüger's hypothesis, 172
 Phenol, 38
 Phenyl-alanine, 21, 48
 Phosphoric acid in nucleic acids, 80, 105-108
 (its mode of combination), 108
 Phyllocyanin, 132
 Phyllootaonin, 132
 Phylloporphyrin, 132
 Phylloxanthine, 132
 Piperazine, 156
 Polyaspartic acid, 156
 Polypeptides, 155-164
 Potassium phthalimide, 57
 Primary albumoses, 141
 Proline, see Pyrrolidine-carboxylic acid
 Propylene bromide, 57
 Propyl-ethylacetoacetate, 135
 Prosthetic groups, 19
 Protalbumose, 144
 Protamines, 54, 65, 67-69
 Proteids, definition of, 19
 Protones, 68
 Pseudo-acids, 3-5
 Pseudo-bases, 3-5
 Pseudo-nucleines, 111
 Pseudo-uric acid, 84, 92
 Purine, 90
 Purine bases, 80 *et seq.*
 Pus cells, 70
 Putrescine, 23, 57
 Pyrimidine bases, 80, 97-102, 103-105
 Pyrochinconic acid, 131
 Pyrotartaric acid, 21, 23
 Pyrrol, 131
 Pyrrolidine-carboxylic acid, 23, 48
 Pyruvic acid, 84

 QUININE, 126

 SALINE precipitants, 10-14
 Salmine, 66
 Scombrine, 66

- Secondary albumoses, 141
 Semi-aldehyde of succinic acid, 39
 phenyl-hydrazide of, 39
 Separation of albumens, 10
 Serine, 22, 32-35, 49
 Skatol, 37
 acetic acid, 37
 Skatol-amino-acetic acid, 38
 Skatol-carbonic acid, 37
 Spermatozoa, 65
 Sturine, 66
 Succinic acid, 59
 Succinimide, 136
 Sulphur bodies derived from albumens,
 27-32
 Synalbumose, 149

TAURINE, 30
 Teichmann's crystals, 125
 Theobromine, 93, 95
 Theophylline, 195
 Thiolactic acid, 29
 α -Thiolactic acid, 31
 β -Thiolactic acid, 31
 Thionuric acid, 84
 Thionyl-chloride, 158
 Thymic acid, 79
 Thymine, 97, 99
 Thymus histone, 71

 Trichlor-lactic acid, 86
 Trichlor-purine, 95
 Tritico-nucleic acid, 101, 109-111
 Tryptophane, 21, 36-41
 Tyrosine, 22, 49

 UNKNOWN base from nucleic acid, 110
 Uracil, 97, 100
 Uramil, 84
 Uramilic acid, 84
 Urea, 59, 86, 99, 100
 Uric acid, 83-96
 Urobilin, 133, 136

 VERWORN'S hypothesis, see "Biogen"
 hypothesis
 Vitellin, 111
 Volatile degradation products, 21

 WITTE'S peptone, 12, 13

XANTHINE, 82, 94
 Xanthoproteid reaction, 8
 Xylic acid, 81
 Xylose, 81

 ZINC sulphate as precipitant, 12, 140
 et seq.

This book should be returned
the Library on the last date stamp
below.

A fine of five cents a day is incurred
by retaining it beyond the specified
time.

Please return promptly.

DUE NOV 30 1914

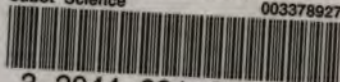
OCT -5 1923

DUE MAY 7 1927

DUE SEP 27 1927

Chem 499.06
Chemistry of the albumens :
Cabot Science

003378927



3 2044 091 929 661